Remarks

In response to the restriction requirement set forth in the Office Action mailed June 6, 2003, applicants identify with traverse the following group for examination: Formula (I), wherein R_1 – R_7 are as found in claim 1 and A is 2,3-dihydrofuryl. Applicants traverse the requirement to limit R_6 and R_7 to only hydrogen and alkyl, but if the Examiner insists, applicants further elect R_6 and R_7 to be independently selected from hydrogen and alkyl. Applicants also traverse the restriction of the embodiments of the A ring, and ask for, at the very least along with the examination of 2,3-dihydrofuryl, the examination of Formula I wherein A is 2,3-dihydrothienyl and 2,3-dihydropyrano, in that order.

Applicants request that examination begin with Example 3 as the elected species. Applicants further respectfully request examination to next proceed on the following species, present in priority of preference: Examples 4, 5, 9 and 10.

With respect to restriction of the method of treatment claims, applicants respectfully request that the Examiner search new claim 30, which recites "[a] method of treating a condition treatable by agonism of the 5HT2 receptor comprising administering a compound according to claim 1." Applicants below present evidence as to why such a claim has unity of invention, therefore rendering restriction improper; however, if the Examiner still deems that restriction with respect to the type of disorder is still proper, applicants elect obesity with traverse.

Applicants traverse the restriction requirement for the reasons discussed below.

US Patent No. 5,633,276

The Examiner cited US Patent No. 5,633,276 to support a finding of lack of unity of invention; however, applicants urge that this reference does not actually support such a finding. The present claims are both novel and unobvious over US '276.

A distinguishable structural feature of the present claims is an indoline group substituted at its nitrogen atom by an aminoethyl group $-(CH_2)(CHR_3)_pNR_1R_2$, wherein R_3 is alkyl; and R_1 and R_2 are H or alkyl. This feature of the present invention is neither taught nor suggested by US '276. In contrast to US '276, the second carbon atom in the sidechain is CH_2, i.e. unsubstituted, and therefore different from the presently claimed compounds.

Another point of distinction is the amino substituents. US '276 requires that one of these substituents be COR^6. In contrast, the amino substituents in the presently claimed compounds are H or alkyl, which are not equivalents or obvious substituents of COR^6. Moreover, the chain length varies according to whether p is 1, 2, 3 or 4, which is in variance with the present invention.

At least in these ways, the present invention is completely different from US '276. Accordingly, applicants respectfully urge that the Examiner is incorrect in stating that the presently claimed compounds lack a structural feature that defines a contribution over the prior art. It is clear that even if one considers the substituted indoline sub-structure in isolation, there is a structural feature, which is the indoline-$(CH_2)(CHR_3)NR_1R_2$ group, wherein R_3 is alkyl; and R_1 and R_2 are H or alkyl.

The R_6 and R_7 groups

With regard to the R_6 and R_7 groups, applicants urge that the Examiner has not shown (i) that there is a lack of unity problem between the various possibilities for R_6 and R_7 or (ii) that the searching of these extra groups would be a serious burden. Applicants note that the fused three ring core compound of Formula I with its aminoethyl side chain serves as the significant structural moiety that is shared by all of the claimed compounds. Accordingly, it is improper to restrict the R_6 and R_7 to groups to anything less than what is recited in claim 1.

Disorders

Applicants have added new claim 30, reciting "[a] method of treating a condition treatable by agonism of the 5HT2 receptor comprising administering a compound according to claim 1." Applicants note that similar wording was recently found acceptable in claim 1 of their recently granted US Patent No. 6,500,866, attached hereto as Appendix I. Newly added method claim 30 has unity of invention and no such method is taught or suggested by US '276. All of the conditions are linked by a single mechanism. Applicants provide further evidence of the unity of invention with respect to the disorders in the attached journal articles in Appendix II. A summary of these articles is presented in Table 1 below.

Table 1

Disease State	***Journal Reference***
depression	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
atypical depression	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
bipolar disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
anxiety disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
obsessive-compulsive disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
social phobias or panic states	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
sleep disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
sexual dysfunction	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
psychoses	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
schizophrenia	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
migraine	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
pain	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
raised intracranial pressure	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
epilepsy	European J. Pharmacol. 359 (1998), 33-40.
personality disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
age-related behavioural disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
behavioural disorders associated with dementia	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
organic mental disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
mental disorders in childhood	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
aggressivity	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
age-related memory disorders	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
chronic fatigue syndrome	Int. J. Fertility and Women's Medicine 42(2) 67-72
drug and alcohol addiction	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
obesity	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
bulimia	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
anorexia nervosa	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362.
premenstrual tension	Int. J. Fertility and Women's Medicine 42(2) 67-72
trauma	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362
stroke	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362
neurodegenerative diseases	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362
encephalitis	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362
meningitis	Curr. Opin. Invest. Drugs (April 1993), 2(4):317-362
thrombosis	J. Pharmacol. Exp. Ther. (1997) 280(2) 761-769
sleep apnea	Neuroscience Lett. (1992) 139, 243-248

Should additional fees be necessary in connection with the filing of this response, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

Respectfully submitted,

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Studies on the role of 5-HT_{2C} and 5-HT_{2B} receptors in regulating generalised seizure threshold in rodents

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Abstract

The present studies were conducted to investigate the role of 5-HT_{2C} and 5-HT_{2B} receptors in the generation of pentylenetetrazol and electroshock-evoked seizures. The 5-$HT_{2C/2B}$ receptor-preferring agonist 1-(*m*-chlorophenyl)-piperazine (mCPP; 2.5–7 mg/kg i.p.) weakly elevated seizure threshold in the mouse (but not the rat) electroshock test and also provided appreciable protection against pentylenetetrazol-induced myoclonic and/or tonic seizures in mice and rats, an action that was inhibited by the 5-$HT_{2C/2B}$ receptor antagonist 5-methyl-1-(3-pyridylcarbomoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB-206553; 10–20 mg/kg p.o.). In contrast, the 5-HT_{2B} receptor agonist 1-[5-(2-thienylmethoxy)-1*H*-3-indoyl]propan-2-amine hydrochloride (BW-723C86; 3–30 mg/kg s.c.) had no effect on the threshold for generalised seizures in any of the models employed. These results indicate that the observed anticonvulsant effects of mCPP are likely to be mediated by activation of 5-HT_{2C} receptors. However, blockade of these receptors in mice (or rats) by SB-206553 (5–20 mg/kg p.o.) did not result in the reduced seizure threshold characteristic of mutant mice deficient of 5-HT_{2C} receptors, suggesting that in normal adult animals this receptor subtype may usually be subjected to only a low level of 5-hydroxytryptamine tone.

Keywords: 5-HT_{2C} receptor; 5-HT_{2B} receptor; SB-206553; BW-723C86; *m*-Chlorophenylpiperazine; Seizure

1. Introduction

There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically epilepsy-prone rodents (Kilian and Frey, 1973; Buterbaugh, 1978; Przegalinski, 1985; Hiramatsu et al., 1987; Dailey et al., 1992). Generally, agents that elevate extracellular serotonin (5-hydroxytryptamine, 5-HT) levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both limbic and generalised seizures (De La Torre et al., 1970; Löscher et al., 1984; Prendiville and Gale, 1993; Yan et al., 1994). Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsions (De La Torre et al., 1970; Browning et al., 1978; Statnick et al., 1996).

Little is known about the role of 5-HT receptor subtypes in the modulation of seizure activity. Recent experiments using pharmacological probes have implicated 5-HT_2 receptors in the development of amygdala-kindled limbic seizures (Wada et al., 1997) and the expression of electrically induced generalised seizures (Przegalinski et al., 1994) in rodents. The possible involvement of 5-HT_{2C} receptors has been suggested by the finding that genetically-modified mice lacking this receptor subtype undergo spontaneous generalised seizures and exhibit a reduced seizure threshold (Tecott et al., 1995).

In order to further delineate the role of 5-HT_{2C} receptors in seizure generation, we have determined the effects of the 5-$HT_{2C/2B}$ receptor-preferring agonist 1-(*m*-chlorophenyl)-piperazine (mCPP) (Kennett, 1993) and the 5-$HT_{2C/2B}$ receptor antagonist 5-methyl-1-(3-pyridylcarbomoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB-206553) (Kennett et al., 1996b) in established models of electrically and chemically induced generalised seizures (Upton, 1994; Upton et al., 1997). For comparative pur-

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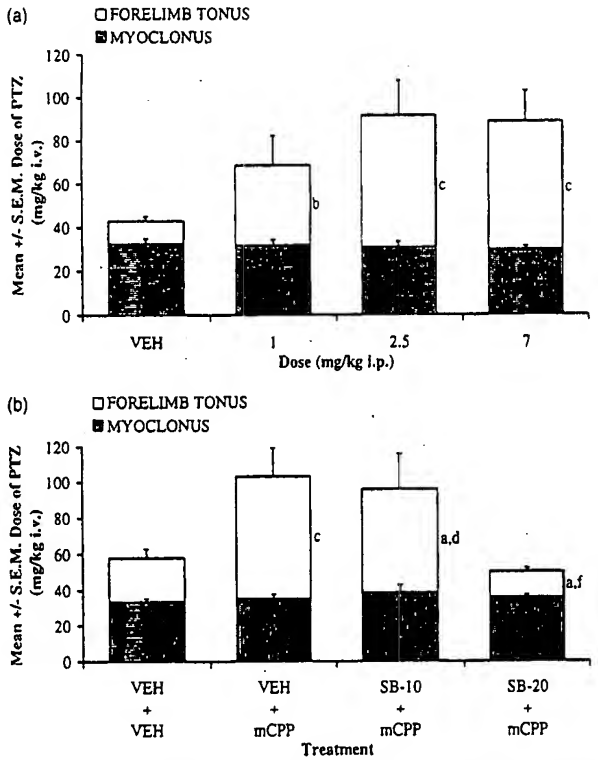

Fig. 2. (a) Anticonvulsant effect of mCPP (1–7 mg/kg i.p., 20 min pre-test) in the rat i.v. pentylenetetrazol (PTZ) infusion test. Data are the mean (± S.E.M.) doses of PTZ required to induce myoclonic and tonic forelimb extension seizures in groups of 11–12 rats. $^{b}P < 0.05$, $^{c}P < 0.01$, compared to vehicle (VEH)-treated controls by (two-tailed) Mann–Whitney *U*-test following significant Kruskal–Wallis ANOVA (myoclonic seizures; $H(df = 3) = 4.01$, non-significant: forelimb tonus; $H(df = 3) = 14.26$, $P < 0.01$). (b) Antagonism of the anticonvulsant effect of mCPP (2.5 mg/kg i.p., 20 min pre-test) by SB-206553 (SB; 10–20 mg/kg p.o., 1 h pre-test). Data are the mean (± S.E.M.) doses of PTZ required to induce myoclonic and tonic forelimb extension seizures in groups of 11–12 rats. ^{a}Non-significant, $^{f}P < 0.01$, compared to vehicle (VEH) controls and ^{d}non-significant, $^{f}P < 0.01$, compared to mCPP alone by (two-tailed) Mann–Whitney *U*-test following significant Kruskal–Wallis ANOVA (forelimb tonus; $H(df = 3) = 9.43$, $P < 0.05$).

single dose. However, SB-206553 (20 mg/kg p.o.) was able to completely inhibit the protective action of mCPP (2.5 mg/kg i.p.) against pentylenetetrazol-induced myoclonic and/or tonic seizures in both mice (Fig. 1b) and rats (Fig. 2b).

4. Discussion

The pentylenetetrazol infusion and maximal electroshock seizure threshold tests employed in the present studies were selected for their sensitivity to both known anticonvulsant (e.g., carbamazepine, diazepam) and proconvulsant (e.g., picrotoxin, 4-aminopyridine, FG-7142) agents and also because they evoke several different types of generalised convulsions (Löscher and Schmidt, 1988; Upton, 1994). In these mouse and/or rat models, the 5-HT_{2B} receptor agonist BW-723C86 (Kennett et al., 1997a) had no effect on the threshold for myoclonic, forelimb tonic (pentylenetetrazol-induced) or hindlimb tonic (pentylenetetrazol or electroshock-induced) seizures. The compound was tested at doses (3–30 mg/kg s.c.) shown previously to evoke central 5-HT_{2B} receptor-mediated hyperphagia (Kennett et al., 1997a) and anxiolysis (Kennett et al., 1996a) in rats. The present findings are in accord with the observation that the selective 5-HT_{2B} receptor antagonist 6-chloro-5-methyl-1-(5-quinolylcarbamoyl)indoline (SB-215505; 1–10 mg/kg p.o.) does not alter seizure threshold in the rat pentylenetetrazol infusion test (data not shown). In contrast, the 5-$HT_{2C/2B}$ receptor-preferring agonist mCPP (Kennett, 1993) weakly elevated seizure threshold in the mouse (but not the rat) maximal electroshock seizure threshold test and also provided appreciable protection against pentylenetetrazol-induced myoclonic and tonic seizures in mice and forelimb tonic seizures in rats. Taken together, the findings with BW-723C86 and mCPP suggest that the anticonvulsant properties of the latter agent are most likely to be attributable to an agonist action at 5-HT_{2C} receptors. This idea is supported by the observation that the 5-$HT_{2C/2B}$ receptor antagonist SB-206553 (Kennett et al., 1996b) was able to completely inhibit the anticonvulsant effects of mCPP (2.5 mg/kg i.p.) in the mouse and rat pentylenetetrazol infusion models at a dose (20 mg/kg p.o.) reported to antagonise other 5-HT_{2C} receptor-mediated functions in vivo (Kennett et al., 1996b).

The ability of mCPP to prevent tonic extension in mice and rats indicates that 5-HT_{2C} receptors may play a role in regulating seizure spread. In mice, mCPP also inhibits myoclonus suggesting an additional role for 5-HT_{2C} receptors in this species of raising seizure threshold (Piredda et al., 1985; Löscher and Schmidt, 1988). Interestingly, the level of anticonvulsant activity produced by mCPP against all seizure types in the mouse pentylenetetrazol infusion test was observed to diminish at the highest dose tested. It is presently unclear whether this decline is related to an action at 5-$HT_{2C/2B}$ receptors or is due to the emergence of effects at other receptor subtypes.

Although activation of 5-HT_{2C} receptors appeared to result in an anticonvulsant action, SB-206553 alone did not lower the threshold to myoclonus, forelimb and/or hindlimb tonus in mice or rats thereby indicating that blockade of this receptor subtype was not associated with enhanced susceptibility to generalised seizures. This finding is consistent with experiments demonstrating that the highly selective 5-HT_{2C} receptor antagonist SB-242084 did not produce proconvulsant activity in the rat maximal electroshock seizure threshold test even after administration at a very high acute dose (30 mg/kg p.o.) (Kennett et al., 1997b).

The inability of 5-HT_{2C} receptor antagonists to reduce seizure threshold in adult rodents contrasts with the observed characteristics of mutant mice lacking the 5-HT_{2C} receptor (Tecott et al., 1995). The mutant mice undergo spontaneous tonic-clonic convulsions and by 2–3 months of age exhibit enhanced susceptibility to pentylenetetrazol and audiogenic-induced seizures (Tecott et al., 1995; Brennan et al., 1997). The present results suggest that the epileptic phenotype exhibited by 5-HT_{2C} receptor-deficient mice may be secondary to developmental or neuroadaptive changes in the brain.

The failure of BW-723C86 to modulate pentylenetetrazol or electroshock-induced myoclonic or tonic extensor convulsions, implies that 5-HT_{2B} receptors are not directly involved in propagating these types of generalised seizures. Activation of 5-HT_{2C} receptors using agents such as mCPP produces an anticonvulsant profile in the pentylenetetrazol and maximal electroshock seizure threshold models indicating that this receptor subtype contributes mainly to the spread of generalised seizures in mice and rats but may also play a role in their induction in the former species. However, blockade of these receptors is not associated with a lowering of seizure threshold suggesting that the 5-HT_{2C} receptors implicated in the regulation of seizure generation and spread may normally be subjected to only a low level of 5-HT tone.

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Neuroactive steroids exacerbate γ-hydroxybutyric acid-induced absence seizures in rats

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Abstract

Certain naturally-occurring steroid metabolites and their synthetic analogs (neuroactive steroids) allosterically enhance $GABA_A$ receptor function and possess potent anticonvulsant properties. In the present study, the effect of two synthetic neuroactive steroids, alphaxalone (5α-pregnane 3α-ol-11, 20-dione) and tetrahydrodeoxycorticosterone was studied in a rat model of generalized absence seizures induced by γ-hydroxybutyric acid. Both steroids dose-dependently exacerbated γ-hydroxybutyric acid-induced absence seizures upon systemic administration and after focal administration into thalamic ventrobasal nucleus. However, alphaxalone and tetrahydrodeoxycorticosterone failed to potentiate γ-hydroxybutyric acid-induced absence seizures when injected into thalamic reticular nucleus. In all the doses of steroids tested in thalamic reticular nucleus, the duration of γ-hydroxybutyric acid-seizures was neither prolonged nor shortened. This nonresponsiveness of thalamic reticular nucleus to neuroactive steroids in modulating absence seizures may have arisen due to the molecular heterogeneity of $GABA_A$ receptor subunits within the thalamus.

Keywords: Absence seizure; Neuroactive steroid; Thalamus; γ-Hydroxybutyric acid; $GABA_A$ receptor

1. Introduction

Generalized absence seizures occur as highly synchronized thalamocortical oscillations (3 Hz) which evolve most readily from thalamic relay nuclei (e.g., ventrobasal nucleus) and the neocortex (Gloor et al., 1990). This oscillatory behavior in the thalamocortical network is regulated by thalamic reticular nucleus (McCormick, 1992), and it is believed that γ-aminobutyric acid (GABA)-ergic inhibition within the thalamus plays an important role in the generation and/or regulation of absence seizures. For example, focal administration of bicuculline (a $GABA_A$ receptor antagonist) into thalamic reticular nucleus has been shown to increase 3 Hz oscillation in thalamic slices (Huguenard and Prince, 1994), while in whole animal studies absence seizures are inhibited by direct injection of muscimol (a $GABA_A$ receptor agonist) into thalamic reticular nucleus. In contrast, focal injection of muscimol in thalamic relay nuclei exacerbates absence seizures (Liu et al., 1991). A similar exacerbation of absence seizures is observed after systemic administration of $GABA_A$ receptor agonists (King, 1979; Vergnes et al., 1984; Smith and Bierkamper, 1990). These findings together suggest that while a generalized increase in $GABA_A$ergic inhibition in the brain (after systemic injection of GABA-mimetics) tends to worsen absence seizures, a more selective increase in $GABA_A$ergic inhibition in thalamic reticular nucleus may attenuate absence seizures.

Low levels of 3α-hydroxy metabolites of progesterone and deoxycorticosterone (neurosteroids) are found in the brain (Paul and Purdy, 1992). These steroid metabolites are known to alter brain excitability, and cause sedation and anesthesia by allosterically enhancing the function of the $GABA_A$ receptors (Majewska et al., 1986; Turner et al., 1988; Morrow et al., 1990). There is some clinical evidence that these naturally-occurring steroid metabolites may possess anticonvulsant activities. For example, in women with partial focal epilepsy, the frequency of seizures during the luteal phase is usually low when the plasma progesterone levels increase (Mellon, 1994). The metabolism of progesterone to allopregnanolone (a 3α-hy-

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Studies on the role of 5-HT_{2C} and 5-HT_{2B} receptors in regulating generalised seizure threshold in rodents

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Abstract

The present studies were conducted to investigate the role of 5-HT_{2C} and 5-HT_{2B} receptors in the generation of pentylenetetrazol and electroshock-evoked seizures. The 5-$HT_{2C/2B}$ receptor-preferring agonist 1-(*m*-chlorophenyl)-piperazine (mCPP; 2.5–7 mg/kg i.p.) weakly elevated seizure threshold in the mouse (but not the rat) electroshock test and also provided appreciable protection against pentylenetetrazol-induced myoclonic and/or tonic seizures in mice and rats, an action that was inhibited by the 5-$HT_{2C/2B}$ receptor antagonist 5-methyl-1-(3-pyridylcarbomoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB-206553; 10–20 mg/kg p.o.). In contrast, the 5-HT_{2B} receptor agonist 1-[5-(2-thienylmethoxy)-1*H*-3-indoyl]propan-2-amine hydrochloride (BW-723C86; 3–30 mg/kg s.c.) had no effect on the threshold for generalised seizures in any of the models employed. These results indicate that the observed anticonvulsant effects of mCPP are likely to be mediated by activation of 5-HT_{2C} receptors. However, blockade of these receptors in mice (or rats) by SB-206553 (5–20 mg/kg p.o.) did not result in the reduced seizure threshold characteristic of mutant mice deficient of 5-HT_{2C} receptors, suggesting that in normal adult animals this receptor subtype may usually be subjected to only a low level of 5-hydroxytryptamine tone.

Keywords: 5-HT_{2C} receptor; 5-HT_{2B} receptor; SB-206553; BW-723C86; *m*-Chlorophenylpiperazine; Seizure

1. Introduction

There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically epilepsy-prone rodents (Kilian and Frey, 1973; Buterbaugh, 1978; Przegalinski, 1985; Hiramatsu et al., 1987; Dailey et al., 1992). Generally, agents that elevate extracellular serotonin (5-hydroxytryptamine, 5-HT) levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both limbic and generalised seizures (De La Torre et al., 1970; Löscher et al., 1984; Prendiville and Gale, 1993; Yan et al., 1994). Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsions (De La Torre et al., 1970; Browning et al., 1978; Statnick et al., 1996).

Little is known about the role of 5-HT receptor subtypes in the modulation of seizure activity. Recent experiments using pharmacological probes have implicated 5-HT_2 receptors in the development of amygdala-kindled limbic seizures (Wada et al., 1997) and the expression of electrically induced generalised seizures (Przegalinski et al., 1994) in rodents. The possible involvement of 5-HT_{2C} receptors has been suggested by the finding that genetically-modified mice lacking this receptor subtype undergo spontaneous generalised seizures and exhibit a reduced seizure threshold (Tecott et al., 1995).

In order to further delineate the role of 5-HT_{2C} receptors in seizure generation, we have determined the effects of the 5-$HT_{2C/2B}$ receptor-preferring agonist 1-(*m*-chlorophenyl)-piperazine (mCPP) (Kennett, 1993) and the 5-$HT_{2C/2B}$ receptor antagonist 5-methyl-1-(3-pyridylcarbomoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB-206553) (Kennett et al., 1996b) in established models of electrically and chemically induced generalised seizures (Upton, 1994; Upton et al., 1997). For comparative pur-

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Keywords: Absence seizure; Neuroactive steroid; Thalamus; γ-Hydroxybutyric acid; $GABA_A$ receptor

1. Introduction

Generalized absence seizures occur as highly synchronized thalamocortical oscillations (3 Hz) which evolve most readily from thalamic relay nuclei (e.g., ventrobasal nucleus) and the neocortex (Gloor et al., 1990). This oscillatory behavior in the thalamocortical network is regulated by thalamic reticular nucleus (McCormick, 1992), and it is believed that γ-aminobutyric acid (GABA)-ergic inhibition within the thalamus plays an important role in the generation and/or regulation of absence seizures. For example, focal administration of bicuculline (a $GABA_A$ receptor antagonist) into thalamic reticular nucleus has been shown to increase 3 Hz oscillation in thalamic slices (Huguenard and Prince, 1994), while in whole animal studies absence seizures are inhibited by direct injection of muscimol (a $GABA_A$ receptor agonist) into thalamic reticular nucleus. In contrast, focal injection of muscimol in thalamic relay nuclei exacerbates absence seizures (Liu et al., 1991). A similar exacerbation of absence seizures is observed after systemic administration of $GABA_A$ receptor agonists (King, 1979; Vergnes et al., 1984; Smith and Bierkamper, 1990). These findings together suggest that while a generalized increase in $GABA_A$ergic inhibition in the brain (after systemic injection of GABA-mimetics) tends to worsen absence seizures, a more selective increase in $GABA_A$ergic inhibition in thalamic reticular nucleus may attenuate absence seizures.

Low levels of 3α-hydroxy metabolites of progesterone and deoxycorticosterone (neurosteroids) are found in the brain (Paul and Purdy, 1992). These steroid metabolites are known to alter brain excitability, and cause sedation and anesthesia by allosterically enhancing the function of the $GABA_A$ receptors (Majewska et al., 1986; Turner et al., 1988; Morrow et al., 1990). There is some clinical evidence that these naturally-occurring steroid metabolites may possess anticonvulsant activities. For example, in women with partial focal epilepsy, the frequency of seizures during the luteal phase is usually low when the plasma progesterone levels increase (Mellon, 1994). The metabolism of progesterone to allopregnanolone (a 3α-hy-

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Mood Disorders in the Female Patient

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ABSTRACT: Disruptive changes in mood and low energy level are among the most common reasons women consult a physician. Usually no clear physiological explanation for these changes can be found. Many physicians feel uncomfortable dealing with patients with these complaints. The purpose of this paper is to discuss a practical approach to helping women with such conditions.

A variety of terms have been utilized to refer to the situation in which a female patient has decreased energy or labile mood. Premenstrual Syndrome (PMS) and chronic fatigue syndrome (CFS) are currently popular terms. An association of low mood with menstrual cycle phase is undoubted, with the late luteal–early premenstrual phase most commonly associated with depression and irritability. It seems likely that women with PMS and those without it do not differ in circulating hormone levels during their cycles but rather in the brain response to these. Estrogen and progesterone receptors exist in the brain and change during the cycle.

Elaborate diagnostic efforts are rarely rewarding in managing mood and energy disorders. Of more value is a careful history particularly concerned with the pattern of mood changes and with life stresses, accompanied by a thorough physical examination and laboratory tests. In most cases, changes in mood and energy are a variant of clinical depression. Changes in energy and sleep may be more evident than low affect. Treatment with an appropriate antidepressant, usually a selective serotonin re-uptake inhibitor (SSRI), benefits most of these patients. Allowing the patient to express concerns about stressful life situations is often of great value. *Int J Fertil* 42(2):67–72, 1997

KEY WORDS: PMS, mood disorders, chronic fatigue syndrome

INTRODUCTION

MANY PATIENTS CONSULT A physician because of concerns about mood changes or lack of energy. These two tend to be found in association, and often are accompanied by one or more of a variety of overlapping complaints, which are discussed below. Lack of energy is one of the most common reasons for patients to consult a physician. Such concerns are often expressed by women patients, although energy disorders also occur in men. Many physicians find these patients frustrating to deal with. However, the majority of them (though not all) can be helped, based on an understanding of the disorder and resistance to pressures to prescribe inappropriate or faddish therapies. The purpose of this article is to outline the clinical features of these conditions, to discuss possible causative factors and, even more important, factors that are commonly held to be causative, but are not.

Physicians and other health professionals are trained to deal with disease, and most have difficulty with patients whose complaints do not conform with a recognizable disease. There are two tendencies in this situation that tend to make the problem worse. The first, and perhaps most common on the part of physicians, is to conclude that no disease is present and so inform the patient. The patient often responds negatively, because she feels her problem is not taken seriously, or that there is an implication that she is deceptive to herself or to others, or both. Often, lack of diagnosable disease in a patient with complaints is taken to indicate psychological causality, that "the problem is all in her head." The other extreme is to create a disease entity as an explanation for the symptoms. Thus, feeling tired and shaky is "hypoglycemia," fatigue and weight gain is "hidden hypothyroidism," malaise is "chronic candidiasis," and so on. Some physicians accept such entities, but more often they are applied by alternative health care practitioners or

journalists. While denial of disease frustrates the patient by denying her own perception that something is wrong, creating a disease tends to encourage maintaining the sick role, with consequent loss of function. Sometimes treatments proposed are disruptive—wheat- and grain-free diets—or unhealthful. Typically, the patient feels helped for a while, then moves on to a different "diagnosis" and different treatment regimen. Some patients spend considerable time looking for an answer and considerable money on questionable treatments.

MOOD DISORDERS AND SYMPTOMATOLOGY IN A SOCIOCULTURAL CONTEXT

It may be useful to consider the sorts of conditions in which impairment of mood and energy is prominent without a clear organic basis, and which occur in otherwise healthy people. Then, an approach to such patients will be proposed that can help many of them. Finally, the use of medication will be briefly reviewed.

Disorders of mood and energy are not entirely separate, but in some affected women one occurs without the other. Sadness is variable. Many state that any depression is the result of the condition rather than the cause, and state that they are quite happy with their lives except for the mood swings and/or lack of energy. Often, the affected woman will be unable to participate in activities she likes because of lack of energy. An otherwise good relationship with husband and children may be threatened by irritability, which to the patient seems inexplicable. Typically, hormones are blamed for this last symptom.

A wide variety of symptoms can be associated with mood and energy disorders. There are many patterns of such symptoms for which terms have been proposed. A partial list of these is found in Table I. Often the disorder is described as new, and as one which most doctors have been unwilling to recognize. Typically, alternative practitioners or physicians become advocates for the existence of a new disorder overlooked by others in their profession. Books appear describing the condition, and sometimes become best sellers. Two examples are *Hypothyroidism, the Silent Illness* and *The Yeast Connection.* Economic interests are often associated with such authorship, though this may of course be true also of quite sound publications for lay readers.

These conditions have some common features.

TABLE I
Some terms used to describe dysphoric states.

Hysteria
Neurasthenia
Fatigue
Lack of energy
Fluid retention
Puffiness
Bloating
PMS
Immune system dysfunction
Chronic fatigue syndrome (CFS)
Chronic candidiasis
Persistent EBV infection
Lyme disease
Multiple Chemical Sensitivity (MCS)
Hypoglycemia
Mild adrenal insufficiency
Hypothyroidism: "The silent illness"
Fibrositis
Interstitial cystitis
Irritable bowel syndrome
Idiopathic edema
"Low blood pressure"
Idiopathic orthostatic hypotension
Clinical depression
Seasonal affective disorder (SAD)

They result in assumption of a sick role in which social function is impaired. There may be job disability or inability to participate in social functions with the family. Sometimes, the spouse accompanies the patient on a prolonged search for a medical explanation and shares the patient's frustration and anger when explanations fail to satisfy. Theories of etiology of these conditions often involve a sense of vulnerability to internal factors, such as hormones, or external ones, such as environmental chemicals. Psychosomatic explanations are not acceptable. Throughout, the patient feels a desire to return to full activity but is unable to do so.

The diseases selected as explanations for the patient's disorder are typically ones with symptoms that are common and nonspecific. Thus, decreased

energy, sluggishness, feeling cold too easily, and weight gain may be features of hypothyroidism; although a surprising number with marked hypothyroidism lack them, it is evident that the overwhelming majority of people with these common complaints do not have thyroid disease. It is difficult for lay people to grasp the idea that one may have symptoms of a disease without having the disease.

The specialist in women's health is most likely to see this group of conditions when symptoms are attributed to PMS or to chronic fatigue. Presentation is often dependent on the physician's specialty, since individuals will choose which physician to consult based on their perception of the nature of the problem. Thus, those who feel their problem is due to hormonal fluctuations will see a gynecologist, while those attributing it to multiple chemical sensitivity will see an allergist. Those with musculoskeletal complaints form an overlapping group with those with mood and energy disorders, although for them pain is perceived as primary, and mood and energy changes as derived from it. They often elect to see a rheumatologist or orthopedist. Some patients change the focus of their symptoms and at one time may be most concerned about mood and at another time about pain.

While it is evident that psychological and personality factors are very important in this group of conditions, it cannot be concluded that physical or organic factors play no role. Many of the listed conditions occur in objective form, for example, hypothyroidism, but many who think they have the condition do not have any confirming features, such as elevated TSH for hypothyroidism, or definite immune dysfunction in chronic fatigue syndrome (CFS). It is likely that subtle hypothyroidism can occur without an elevated TSH, or immune defects without definite laboratory confirmation. The problem is that most individuals who think they have these conditions almost certainly do not, and treatment directed at incorrect etiology will obviously be unsuccessful as well as potentially harmful.

Premenstrual syndrome also occupies a middle ground between organic and functional. There is no doubt that many women feel low mood and become irritable in the late luteal phase. However, the diagnosis of PMS requires something more than mild dysphoria. Some women handle the premenstrual state without particular difficulty; others do not. The most important issue, therefore, is what distinguishes women who feel they cannot cope with it—those who feel that their work or close relationships are disrupted by their premenstrual difficulties. Careful history taking is vital. The great majority of women who regard themselves as having PMS do not have their dysphoria confined to the premenstrum. However, it may be worse then or occur most often then. Such women have premenstrual exacerbation of more pervasive mood problems. They may be able to restrain their feelings and behavior during the rest of the cycle but find themselves at the edge of control late luteally.

There is no doubt that some women have dysphoria that is confined to the premenstrual phase, despite controversies about the concept of PMS [1]. This is seen commonly as a result of hormone replacement regimens that utilize medroxyprogesterone acetate (MPA). Some women who have not previously experienced premenstrual dysphoria do so with MPA. It is far less common with micronized progesterone, but can occur. The fact that PMS may be induced by exogenous progestins in women who have not previously been troubled by it is incontrovertible evidence that the syndrome can have an organic basis. However, PMS is usually combined with factors less objective.

It is most useful to regard PMS as a mixture of organic and psychosocial factors. It is not necessarily productive to try to tease out these separate factors in each case, but rather work to help with both.

PMS is a variant of depression that is precipitated or exacerbated by hormonal events. While Dalton [2] popularized the idea that PMS is due to inadequate late luteal progesterone levels, studies have not confirmed differences in hormone levels between women with PMS and those without it. This is not surprising. The brain is an important target organ for hormone action, and this is certainly true for sex steroids. What is distinctive in PMS is not the circulating hormone levels but the brain's response to these hormones. There are many other examples of this in classical endocrinology. For example, growth of the breast is stimulated by estrogen, yet variation in breast size is due not to differences in estrogen levels but to end-organ differences. The exact mechanism by which the hormonal events of the cycle evoke mood changes is unknown; it is difficult to study brain tissue responses in humans, and animal models for a condition like PMS are problematic.

It is useful to regard PMS as a form of depression modulated by cyclic hormone changes. This implies

that medications useful for depression may be useful for PMS, and this is in fact the case. However, the concept of depression is not so simple as it appears, and needs to be examined. Many women with PMS, as well as those with the other conditions listed in Table I, do not regard themselves as depressed and often object vigorously to being so labeled.

In medical usage, *depression* refers to a clinical syndrome characterized by low mood, decreased energy, pessimism, and so-called vegetative signs (see Table II). In lay usage it refers to feelings of sadness or low mood. With the success of the selective serotonin reuptake inhibitors (SSRIs), depression is increasingly viewed as a biochemical disturbance in the brain rather than in psychodynamic terms. This is particularly true in medicine, but less among non-health professionals. Once depression is conceived as a biochemical neuro-regulatory disorder, it is not surprising that individuals may be found to have the biochemical disturbance—as evidenced by clinical response to SSRIs—but not all of the clinical features. This is commonly the case with women coming to a physician with complaints of PMS or decreased energy. They may have the low energy and decreased enthusiasm of depression without an inner feeling of being depressed. Recognizing this can avoid many arguments with patients who will accept the diagnosis of PMS but not that of depression. Many who admit to mood swings and even frequent tearfulness will deny depression or sadness.

Depression is stigmatized in our culture. Accepting that one is depressed is difficult for both men and women, but in somewhat different ways. Men perhaps worry that they are "losers" if they are not happy. For women, a variety of other feelings come into play. Many women to whom I have hinted that their problems might be a form of depression give a reply something like this: "But I have a wonderful husband, a nice house, two healthy children." To be depressed in this context seems to imply ingratitude. Also many women have grown up feeling that they should please others. They do this by smiling, dressing attractively, helping in school or community activities, and the like. To acknowledge depression is to admit a limit to being able to please others and to face the possibility of needs that have not been served in a life focused, at least consciously, on the wants of others. Whether because of biological or psychosocial factors or both, women seem to be more vulnerable to depression than men; prompt recognition is important [3].

TABLE II
Clinical depression.

Clinical depression is diagnosed on the basis of:
Low affect: sadness, despondency, hopelessness, anhedonia.
Vegetative signs: psychomotor retardation, anorexia, low energy, insomnia, constipation. Some have endocrine changes that are probably epiphenomena.

However, this classical description does not fit many depressed patients:
- Different presentation to different specialties

Younger patients usually do not have vegetative signs:
- Many gain rather than lose weight.
- Sad affect is often not apparent.

Distinction between endogenous and exogenous depression is often—but not always—problematic.

Against the reluctance of Americans to admit to being depressed must be balanced the widespread use of SSRIs. Faces in advertisements seem all to be smiling, but for everyone at times the reality of life is different. This is not the place to speculate on why depression is so common in our culture and yet so widely denied. However, the physician must be sensitive to this paradox to work successfully with those many patients with features of depression who are distressed by this diagnostic label.

It is not always the case that chronic fatigue, PMS, and the many other conditions listed in Table I are simply depression. While depression is a feature of most or all of them, there may be other factors as well (Table III). Most often they are the interaction of physical and psychosocial factors. Organic disease must be ruled out first. This does not mean, however, that elaborate laboratory testing or visits to multiple specialists are usually helpful or always necessary [4]. Often, a careful history is sufficient and limited laboratory testing—TSH for hypothyroidism, for example—is all that is necessary or appropriate. Occasionally, serious organic disease must be ruled out. Immunological testing is not generally helpful in chronic fatigue syndrome unless there is actual evidence of recurrent infection. Infection in this context refers to serious or unusual infections, not frequent upper respiratory infections.

TABLE III
Causes of mood/energy problems.

Biochemical—serotonin insufficiency

Life events
Life style
High level of demand
Unrealistic self-expectations/Lack of priority setting
Perfectionism

Depression
Hypochondriasis
Character disorder

Late capitalist society
Spiritual malaise

Life style
Inadequate sleep
Skipping meals ("hypoglycemia")
Low water intake
High salt/fat diet
Caffeine
Alcohol
Smoking
Lack of exercise
Not setting priorities

TREATMENT

Organic or pharmacological factors must be dealt with first. When use of MPA is the cause of PMS, for example (this is usually quite evident from the medical history), the patient can be switched to micronized progesterone, or at least use MPA only every 2 or 3 months. There are a few other drugs, such as beta blockers, which seem to contribute to low energy or mood. Substituting a different class of agent may be useful when practicable.

Life style factors must be addressed also. Heavy use of caffeine seems to result in less stable mood, as well as sleep disturbance, which itself can contribute to low mood and irritability. Heavy alcohol use can impair mood, but this is best dealt with as a substance problem. Regular aerobic exercise is the best nonpharmacological treatment for low mood; yet some affected women work out regularly. Others feel they cannot because of their low energy.

Interpersonal factors are important. For example, a patient came to me at her husband's suggestion because he found her to be irritable with him. When I was taking her history, I noticed some reticence concerning her relationship with her husband. Finally, she said that her husband had only recently come home from federal prison where he had served a 10-year sentence for narcotic smuggling. Obviously, the nonhormonal factors were the decisive ones here. Yet, obvious as it seems, the relationship between the problems with her husband and her mood had not been apparent to the patient. Other cases are less obvious, of course.

While psychotherapy is appropriate for many women with the kinds of conditions discussed here, a majority of those consulting an organically based specialist are unwilling to go for counseling. It is then up to the physician to offer the best help his or her skills allow. Most often this is office counseling combined with antidepressant medication. Antidepresssants are listed in Table IV, and major adverse effects in Table V.

The majority of patients presenting with fatigue, PMS, or mood swings will respond to an SSRI. The idea must be presented tactfully. It is helpful to reassure the patient that the medication will not make her high or euphoric but, rather, restore normal mood and energy, and that it is nonaddictive. For legitimate reasons, there is in our society great fear of mood-altering drugs, and the way antidepressants differ from abusable drugs must often be briefly explained. It is also important to inform the patient that the medication acts gradually and that she will not feel any different after taking the first few pills. A follow-up in 4 to 6 weeks to review the situation and offer reassurance is important.

There are some women with the disorders discussed here who do not respond to SSRIs. Some are very anxious, and thus are afraid to persevere with the medication. Others have character disorders [5], a group of conditions which generally do not benefit from psychotropic medication. Finally, there are probably some in whom the problem is due to different central neural mechanisms, as yet not understood. If the first SSRI tried does not produce benefit despite being raised to the full dose, another may be tried. More complex problems involving multiple psychotropic medications or severe depression with suicidal concerns should be referred to a psychiatrist.

Reassurance is very important for women with the group of conditions described here. Frequently, they ask over and over what is wrong, or seek reassurance that serious disease is not present. This is

TABLE IV
Treatments for mood/energy problems.

I. Life style change
II. Counseling/psychotherapy
Reassurance
III. "Minor tranquilizers"
diazepam (Valium)
chlordiazepoxide (Librium)
alprazolam (Xanax)
chlorazepate (Traxene)
triazolam (Halcion)
buspirone (Buspar)

IV. Tricyclic antidepressants
imipramine (Tofranil)
amitriptyline (Elavil)
desipramine (Norpramin)

Problems: latency, somnolence, weight gain, arrhythmias

V. Other antidepressants
trazodone (Desyrel)
lithium

VI. Selective serotonin reuptake inhibitors (SSRIs)
sertraline (Zoloft) 50–200 mg daily (Paxil)
fluvoxamine (Luvox) 50–300 mg daily
fluoxitine (Prozac) 20–60 mg daily

VII. Serotonin norepinephrine reuptake inhibitors (SNRIs)
venlafaxine (Effexor) 75–275 mg daily,
divided into 2 or 3 doses
nefazodone (Serzone) 100–300 mg b.i.d.

TABLE V
Adverse effects of selective serotonin reuptake inhibitors (SSRIs).

Patient preconceptions
Shame felt at needing antidepressant
Fear due to inaccurate media coverage

Nausea, diarrhea
Stimulation—anxiety
Tiredness
Decreased libido
Sometimes useful to start at a low dose and increase over days to weeks

"Serotonin syndrome" rare—associated with use of SSRI and anti-migraine agents, with agitation, hypomania, disorientation, movement abnormalities and other abnormalities

Seizures—bupropion (Wellbutrin)in 0.4%
Interaction with MAO inhibitors

Induction of cytochrome P-450 system
Inhibition of cytochrome P-450 IIIa4
Displacement of protein-bound drugs

often irritating to physicians who feel the patient has not listened to the explanation. Physicians can underestimate how comforting it is to the patient to be told once again that there is no serious underlying disease. Many patients with these conditions will be best off with relatively frequent visits, at least at first, to give repeated reassurance.

CONCLUSION

This article has outlined an approach to a group of disorders that women's health providers encounter often. By avoiding getting caught up in the theoretical complexities, ruling out plausible organic etiology, and a combination of reassurance and medication, physicians can help most of these women, although there will remain a few whom we find ourselves unable to help despite our best efforts.

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Affective Spectrum Disorders: How to Recognize and Treat Depression

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ABSTRACT: Depression often goes undiagnosed. Even when pharmacotherapy is initiated, many patients discontinue therapy and thus risk relapse. Depression may occur at any age; however, the average age of onset is the late twenties. Acronyms have been developed to help the clinician recognize depression in the clinical setting. Common medications, abused substances, and medical disorders may cause and/or mimic depression. If pharmacotherapy is deemed appropriate, the choice of antidepressant is based on personal/family history of response to a particular agent and the side effect profile of the agent, as well as suicide risk. The tricyclic antidepressants and monoamine oxidase inhibitors are associated with anticholinergic effects, orthostasis, and risk of death in overdose. The selective serotonin reuptake inhibitors may have more tolerable adverse effects. Newer agents have also been marketed; however, the selective serotonin reuptake inhibitors are generally the drugs of first choice. *Int J Fertil* 42(2):73–77, 1997

KEY WORDS: depression, pharmacotherapy, diagnosis, review

INTRODUCTION

ONE OF THE MOST COMMON clinical situations faced by primary care clinicians is depression. The actual incidence of major depression is not known; however, it may approximate a lifetime prevalence rate of 5.8% according to the National Institute of Mental Health Epidemiologic Catchment Area study [1]. Furthermore, the incidence rates appear to be increasing.

Depression is two to three times as likely to occur in a woman [2–4]. The highest incidence is between ages 25 and 44, although depression may occur at any time [3,4]. There also appears to be a genetic component, with first-degree relatives of depressed persons as well as twin studies showing a higher likelihood of depression when compared with the general population [5].

Although the term "depression" may connote differences in definitions, *major depressive disorder* is a well-defined term. The *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, defines a major depressive disorder (see Table I). A convenient acronym for the diagnostic criteria is D-SIG E CAPS, which stands for: D depressed mood, S sleep changes, I interest, G guilt, E energy level, C concentration changes, A appetite changes, P psychomotor changes, S suicidal ideation.

It is important for the clinician to recognize the symptoms of depression. Since depression is so common, it would be unlikely for a primary care clinician not to diagnose the disorder on a weekly basis. Undiagnosed depressive disorders are costly to patients, their families, and society. The morbidity, and perhaps mortality, associated with depression is also high [6,7]. For instance, depressed patients miss work days, are high users of medical services, and

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Mood Disorders in the Female Patient

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ABSTRACT: Disruptive changes in mood and low energy level are among the most common reasons women consult a physician. Usually no clear physiological explanation for these changes can be found. Many physicians feel uncomfortable dealing with patients with these complaints. The purpose of this paper is to discuss a practical approach to helping women with such conditions.

A variety of terms have been utilized to refer to the situation in which a female patient has decreased energy or labile mood. Premenstrual Syndrome (PMS) and chronic fatigue syndrome (CFS) are currently popular terms. An association of low mood with menstrual cycle phase is undoubted, with the late luteal–early premenstrual phase most commonly associated with depression and irritability. It seems likely that women with PMS and those without it do not differ in circulating hormone levels during their cycles but rather in the brain response to these. Estrogen and progesterone receptors exist in the brain and change during the cycle.

Elaborate diagnostic efforts are rarely rewarding in managing mood and energy disorders. Of more value is a careful history particularly concerned with the pattern of mood changes and with life stresses, accompanied by a thorough physical examination and laboratory tests. In most cases, changes in mood and energy are a variant of clinical depression. Changes in energy and sleep may be more evident than low affect. Treatment with an appropriate antidepressant, usually a selective serotonin re-uptake inhibitor (SSRI), benefits most of these patients. Allowing the patient to express concerns about stressful life situations is often of great value. *Int J Fertil* 42[2]:67–72, 1997

KEY WORDS: PMS, mood disorders, chronic fatigue syndrome

INTRODUCTION

MANY PATIENTS CONSULT A physician because of concerns about mood changes or lack of energy. These two tend to be found in association, and often are accompanied by one or more of a variety of overlapping complaints, which are discussed below. Lack of energy is one of the most common reasons for patients to consult a physician. Such concerns are often expressed by women patients, although energy disorders also occur in men. Many physicians find these patients frustrating to deal with. However, the majority of them (though not all) can be helped, based on an understanding of the disorder and resistance to pressures to prescribe inappropriate or faddish therapies. The purpose of this article is to outline the clinical features of these conditions, to discuss possible causative factors and, even more important, factors that are commonly held to be causative, but are not.

Physicians and other health professionals are trained to deal with disease, and most have difficulty with patients whose complaints do not conform with a recognizable disease. There are two tendencies in this situation that tend to make the problem worse. The first, and perhaps most common on the part of physicians, is to conclude that no disease is present and so inform the patient. The patient often responds negatively, because she feels her problem is not taken seriously, or that there is an implication that she is deceptive to herself or to others, or both. Often, lack of diagnosable disease in a patient with complaints is taken to indicate psychological causality, that "the problem is all in her head." The other extreme is to create a disease entity as an explanation for the symptoms. Thus, feeling tired and shaky is "hypoglycemia," fatigue and weight gain is "hidden hypothyroidism," malaise is "chronic candidiasis," and so on. Some physicians accept such entities, but more often they are applied by alternative health care practitioners or

that medications useful for depression may be useful for PMS, and this is in fact the case. However, the concept of depression is not so simple as it appears, and needs to be examined. Many women with PMS, as well as those with the other conditions listed in Table I, do not regard themselves as depressed and often object vigorously to being so labeled.

In medical usage, *depression* refers to a clinical syndrome characterized by low mood, decreased energy, pessimism, and so-called vegetative signs (see Table II). In lay usage it refers to feelings of sadness or low mood. With the success of the selective serotonin reuptake inhibitors (SSRIs), depression is increasingly viewed as a biochemical disturbance in the brain rather than in psychodynamic terms. This is particularly true in medicine, but less among non-health professionals. Once depression is conceived as a biochemical neuro-regulatory disorder, it is not surprising that individuals may be found to have the biochemical disturbance—as evidenced by clinical response to SSRIs—but not all of the clinical features. This is commonly the case with women coming to a physician with complaints of PMS or decreased energy. They may have the low energy and decreased enthusiasm of depression without an inner feeling of being depressed. Recognizing this can avoid many arguments with patients who will accept the diagnosis of PMS but not that of depression. Many who admit to mood swings and even frequent tearfulness will deny depression or sadness.

Depression is stigmatized in our culture. Accepting that one is depressed is difficult for both men and women, but in somewhat different ways. Men perhaps worry that they are "losers" if they are not happy. For women, a variety of other feelings come into play. Many women to whom I have hinted that their problems might be a form of depression give a reply something like this: "But I have a wonderful husband, a nice house, two healthy children." To be depressed in this context seems to imply ingratitude. Also many women have grown up feeling that they should please others. They do this by smiling, dressing attractively, helping in school or community activities, and the like. To acknowledge depression is to admit a limit to being able to please others and to face the possibility of needs that have not been served in a life focused, at least consciously, on the wants of others. Whether because of biological or psychosocial factors or both, women seem to be more vulnerable to depression than men; prompt recognition is important [3].

TABLE II
Clinical depression.

Clinical depression is diagnosed on the basis of:
Low affect: sadness, despondency, hopelessness, anhedonia.
Vegetative signs: psychomotor retardation, anorexia, low energy, insomnia, constipation. Some have endocrine changes that are probably epiphenomena.

However, this classical description does not fit many depressed patients:
- Different presentation to different specialties

Younger patients usually do not have vegetative signs:
- Many gain rather than lose weight.
- Sad affect is often not apparent.

Distinction between endogenous and exogenous depression is often—but not always—problematic.

Against the reluctance of Americans to admit to being depressed must be balanced the widespread use of SSRIs. Faces in advertisements seem all to be smiling, but for everyone at times the reality of life is different. This is not the place to speculate on why depression is so common in our culture and yet so widely denied. However, the physician must be sensitive to this paradox to work successfully with those many patients with features of depression who are distressed by this diagnostic label.

It is not always the case that chronic fatigue, PMS, and the many other conditions listed in Table I are simply depression. While depression is a feature of most or all of them, there may be other factors as well (Table III). Most often they are the interaction of physical and psychosocial factors. Organic disease must be ruled out first. This does not mean, however, that elaborate laboratory testing or visits to multiple specialists are usually helpful or always necessary [4]. Often, a careful history is sufficient and limited laboratory testing—TSH for hypothyroidism, for example—is all that is necessary or appropriate. Occasionally, serious organic disease must be ruled out. Immunological testing is not generally helpful in chronic fatigue syndrome unless there is actual evidence of recurrent infection. Infection in this context refers to serious or unusual infections, not frequent upper respiratory infections.

TABLE III
Causes of mood/energy problems.

Biochemical—serotonin insufficiency

Life events
Life style
High level of demand
Unrealistic self-expectations/Lack of priority setting
Perfectionism

Depression
Hypochondriasis
Character disorder

Late capitalist society
Spiritual malaise

Life style
Inadequate sleep
Skipping meals ("hypoglycemia")
Low water intake
High salt/fat diet
Caffeine
Alcohol
Smoking
Lack of exercise
Not setting priorities

TREATMENT

Organic or pharmacological factors must be dealt with first. When use of MPA is the cause of PMS, for example (this is usually quite evident from the medical history), the patient can be switched to micronized progesterone, or at least use MPA only every 2 or 3 months. There are a few other drugs, such as beta blockers, which seem to contribute to low energy or mood. Substituting a different class of agent may be useful when practicable.

Life style factors must be addressed also. Heavy use of caffeine seems to result in less stable mood, as well as sleep disturbance, which itself can contribute to low mood and irritability. Heavy alcohol use can impair mood, but this is best dealt with as a substance problem. Regular aerobic exercise is the best nonpharmacological treatment for low mood; yet some affected women work out regularly. Others feel they cannot because of their low energy.

Interpersonal factors are important. For example, a patient came to me at her husband's suggestion because he found her to be irritable with him. When I was taking her history, I noticed some reticence concerning her relationship with her husband. Finally, she said that her husband had only recently come home from federal prison where he had served a 10-year sentence for narcotic smuggling. Obviously, the nonhormonal factors were the decisive ones here. Yet, obvious as it seems, the relationship between the problems with her husband and her mood had not been apparent to the patient. Other cases are less obvious, of course.

While psychotherapy is appropriate for many women with the kinds of conditions discussed here, a majority of those consulting an organically based specialist are unwilling to go for counseling. It is then up to the physician to offer the best help his or her skills allow. Most often this is office counseling combined with antidepressant medication. Antidepresssants are listed in Table IV, and major adverse effects in Table V.

The majority of patients presenting with fatigue, PMS, or mood swings will respond to an SSRI. The idea must be presented tactfully. It is helpful to reassure the patient that the medication will not make her high or euphoric but, rather, restore normal mood and energy, and that it is nonaddictive. For legitimate reasons, there is in our society great fear of mood-altering drugs, and the way antidepressants differ from abusable drugs must often be briefly explained. It is also important to inform the patient that the medication acts gradually and that she will not feel any different after taking the first few pills. A follow-up in 4 to 6 weeks to review the situation and offer reassurance is important.

There are some women with the disorders discussed here who do not respond to SSRIs. Some are very anxious, and thus are afraid to persevere with the medication. Others have character disorders [5], a group of conditions which generally do not benefit from psychotropic medication. Finally, there are probably some in whom the problem is due to different central neural mechanisms, as yet not understood. If the first SSRI tried does not produce benefit despite being raised to the full dose, another may be tried. More complex problems involving multiple psychotropic medications or severe depression with suicidal concerns should be referred to a psychiatrist.

Reassurance is very important for women with the group of conditions described here. Frequently, they ask over and over what is wrong, or seek reassurance that serious disease is not present. This is

TABLE IV
Treatments for mood/energy problems.

I. Life style change
II. Counseling/psychotherapy
Reassurance
III. "Minor tranquilizers"
diazepam (Valium)
chlordiazepoxide (Librium)
alprazolam (Xanax)
chlorazepate (Traxene)
triazolam (Halcion)
buspirone (Buspar)

IV. Tricyclic antidepressants
imipramine (Tofranil)
amitriptyline (Elavil)
desipramine (Norpramin)

Problems: latency, somnolence, weight gain, arrhythmias

V. Other antidepressants
trazodone (Desyrel)
lithium

VI. Selective serotonin reuptake inhibitors (SSRIs)
sertraline (Zoloft) 50–200 mg daily (Paxil)
fluvoxamine (Luvox) 50–300 mg daily
fluoxitine (Prozac) 20–60 mg daily

VII. Serotonin norepinephrine reuptake inhibitors (SNRIs)
venlafaxine (Effexor) 75–275 mg daily,
divided into 2 or 3 doses
nefazodone (Serzone) 100–300 mg b.i.d.

TABLE V
Adverse effects of selective serotonin reuptake inhibitors (SSRIs).

Patient preconceptions
Shame felt at needing antidepressant
Fear due to inaccurate media coverage

Nausea, diarrhea
Stimulation—anxiety
Tiredness
Decreased libido
Sometimes useful to start at a low dose and increase over days to weeks

"Serotonin syndrome" rare—associated with use of SSRI and anti-migraine agents, with agitation, hypomania, disorientation, movement abnormalities and other abnormalities

Seizures—bupropion (Wellbutrin)in 0.4%
Interaction with MAO inhibitors

Induction of cytochrome P-450 system
Inhibition of cytochrome P-450 IIIa4
Displacement of protein-bound drugs

often irritating to physicians who feel the patient has not listened to the explanation. Physicians can underestimate how comforting it is to the patient to be told once again that there is no serious underlying disease. Many patients with these conditions will be best off with relatively frequent visits, at least at first, to give repeated reassurance.

CONCLUSION

This article has outlined an approach to a group of disorders that women's health providers encounter often. By avoiding getting caught up in the theoretical complexities, ruling out plausible organic etiology, and a combination of reassurance and medication, physicians can help most of these women, although there will remain a few whom we find ourselves unable to help despite our best efforts.

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Affective Spectrum Disorders: How to Recognize and Treat Depression

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ABSTRACT: Depression often goes undiagnosed. Even when pharmacotherapy is initiated, many patients discontinue therapy and thus risk relapse. Depression may occur at any age; however, the average age of onset is the late twenties. Acronyms have been developed to help the clinician recognize depression in the clinical setting. Common medications, abused substances, and medical disorders may cause and/or mimic depression. If pharmacotherapy is deemed appropriate, the choice of antidepressant is based on personal/family history of response to a particular agent and the side effect profile of the agent, as well as suicide risk. The tricyclic antidepressants and monoamine oxidase inhibitors are associated with anticholinergic effects, orthostasis, and risk of death in overdose. The selective serotonin reuptake inhibitors may have more tolerable adverse effects. Newer agents have also been marketed; however, the selective serotonin reuptake inhibitors are generally the drugs of first choice. *Int J Fertil* 42[2]:73–77, 1997

KEY WORDS: depression, pharmacotherapy, diagnosis, review

INTRODUCTION

ONE OF THE MOST COMMON clinical situations faced by primary care clinicians is depression. The actual incidence of major depression is not known; however, it may approximate a lifetime prevalence rate of 5.8% according to the National Institute of Mental Health Epidemiologic Catchment Area study [1]. Furthermore, the incidence rates appear to be increasing.

Depression is two to three times as likely to occur in a woman [2–4]. The highest incidence is between ages 25 and 44, although depression may occur at any time [3,4]. There also appears to be a genetic component, with first-degree relatives of depressed persons as well as twin studies showing a higher likelihood of depression when compared with the general population [5].

Although the term "depression" may connote differences in definitions, *major depressive disorder* is a well-defined term. The *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, defines a major depressive disorder (see Table I). A convenient acronym for the diagnostic criteria is D-SIG E CAPS, which stands for: D depressed mood, S sleep changes, I interest, G guilt, E energy level, C concentration changes, A appetite changes, P psychomotor changes, S suicidal ideation.

It is important for the clinician to recognize the symptoms of depression. Since depression is so common, it would be unlikely for a primary care clinician not to diagnose the disorder on a weekly basis. Undiagnosed depressive disorders are costly to patients, their families, and society. The morbidity, and perhaps mortality, associated with depression is also high [6,7]. For instance, depressed patients miss work days, are high users of medical services, and

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Differential Involvement of Serotonin 2A/C and Thromboxane A_2/Prostanoid Receptors in High- *vs.* Low-Shear Rate Arterial Thrombosis in Rabbits[1]

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ABSTRACT

Experiments performed in 226 pentobarbitone-anesthetized rabbits were designed to investigate the involvement of thromboxane/prostanoid and 5-hydroxytryptamine (5-HT)$_{2A/C}$ receptors during arterial thrombus formation in distinct low- and high-shear rate thrombosis models. Antithrombotic activities of the thromboxane/prostanoid receptor antagonist SQ 29,548 and two chemically distinct 5-HT$_{2A/C}$ receptor antagonists, ritanserin and ketanserin, were assessed first in low-shear rate (~600 sec^{-1}) arterial thrombosis, produced by insertion of a silk thread as thrombogenic substrate into the central section of an extracorporeal arteriovenous shunt established between the left carotid artery and the right jugular vein (n = 77), and second in high-shear rate (~40,000 sec^{-1}) arterial thrombosis, produced by critical stenosis and local endothelial injury of a carotid artery, characterized by cyclic flow reductions (CFRs) due to recurrent platelet aggregation and subsequent dislodgement of the thrombus (n = 149). Under low shear rate, SQ 29,548 (10–2500 μg/kg plus 10–2500 μg/kg/hr i.v.), but not ritanserin or ketanserin (both at 2500 μg/kg i.v.), dose-dependently inhibited thrombus formation. In contrast, under high shear rate, SQ 29,548 (10–160 μg/kg plus 10–160 μg/kg/hr i.v.) and both ritanserin and ketanserin (both at 10–2500 μg/kg i.v.) dose-dependently reduced CFR frequency, with ID$_{50}$ values of 35 μg/kg (95% confidence limits, 24–58 μg/kg), 77 μg/kg (95% confidence limits, 40–132 μg/kg) and 89 μg/kg (95% confidence limits, 36–285 μg/kg) i.v., respectively. Furthermore, local infusion of the stable thromboxane A_2 analog U-46619 (0.63 μg/kg/min) or 5-HT (20.8 μg/kg/min) proximal to the site of injury and stenosis in rabbits pretreated with either SQ 29,548 (40 μg/kg plus 40 μg/kg/hr i.v.) or ritanserin (160 μg/kg i.v.), respectively, restored CFR frequency to vehicle group levels in animals whose CFR frequency was previously reduced. The inhibitory activity of ketanserin and ritanserin on CFRs could not be attributed to 5-HT$_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties or to any hypotensive activity. These results provide firm evidence that thromboxane/prostanoid receptors are involved in arterial thrombosis in rabbits independently of the shear rate, whereas 5-HT$_{2A/C}$ receptors play a major role only in high-shear rate thrombus formation.

Platelet aggregation is influenced by shear forces (Ruggeri, 1994). In particular, GP IIb/IIIa, the final common pathway of platelet aggregation, interacts only with fibrinogen in a low-shear rate environment, whereas it interacts mainly with vWf in a high-shear rate environment (Ruggeri, 1994). Platelet activation plays an important role in arterial thrombosis (Badimon *et al.*, 1992) because, early in the formation of the hemostatic plug, platelet aggregates are formed at the site of vessel injury, bifurcations or stenoses, which present local increases in shear rates (Goldsmith and Turitto, 1986; Strony *et al.*, 1993). To mimic pathophysiological conditions, Folts developed a model of CFRs in critically stenotic canine coronary artery with endothelial damage, thereby producing high-shear rate thrombosis (Folts *et al.*, 1976; Folts, 1995). Ashton *et al.* (1987, 1989) and Golino *et al.* (1989, 1990) showed that both TxA_2 and 5-HT mediated CFRs in this canine model, *via* 5-HT$_2$ and TP receptor activation, respectively. Furthermore, elevated blood levels and tissue concentrations of TxA_2 and 5-HT have been detected around the stenosis and in the distal canine coronary arterial blood (Schmitz *et al.*, 1985; Ashton *et al.*, 1986). However, the roles of 5-HT$_{2A/C}$ and TP receptors in promoting thrombosis under low-shear rate situations are less well documented (Maffrand *et al.*, 1988; Ruggeri, 1994).

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ABBREVIATIONS: CFR, cyclic flow reduction; COX, cyclooxygenase; GP, glycoprotein; HR, heart rate; 5-HT, 5-hydroxytryptamine; I.D., internal diameter; MAP, mean arterial pressure; NS, not significant; SQ 29,548, [1S-[1α,2α(5Z),3α,4α]]-7-[3-[[2-(phenylaminocarbonyl)hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; TP receptor, thromboxane A_2/prostanoid receptor; TxA_2, thromboxane A_2; U-46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$; vWf, von Willebrand factor.

TABLE 2

Influence of drugs on thrombotic occlusion time and hemodynamic parameters in the arteriovenous shunt model

Values are mean ± S.E.M.

Number	Body Weight	Treatment	Dose	Occlusion Time	MAP		HR	
					Base line	Absolute Changes[a]	Base line	Absolute Changes[a]
	kg		μg/kg	min	mm Hg		beats/min	
27	2.5 ± 0.1	Vehicle	1 ml/kg	13.7 ± 1.3	88 ± 3	−9 ± 2	284 ± 6	2 ± 4
4	2.5 ± 0.1	SQ 29,548	10	15.5 ± 1.8	ND[b]	ND	ND	ND
4	2.5 ± 0.1	SQ 29,548	40	17.6 ± 3.3	90 ± 8	−8 ± 3	297 ± 14	−1 ± 12
8	3.0 ± 0.1	SQ 29,548	160	22.2 ± 2.8*	94 ± 7	−11 ± 3	307 ± 15	−14 ± 4
8	2.8 ± 0.1	SQ 29,548	630	22.5 ± 4.2*	86 ± 3	−7 ± 4	298 ± 14	−10 ± 8
5	2.8 ± 0.2	SQ 29,548	2500	31.3 ± 7.3*	85 ± 6	−15 ± 5	294 ± 22	−2 ± 6
8	2.1 ± 0.1	Ritanserin	2500	14.7 ± 1.6	75 ± 4	−3 ± 3	281 ± 12	−15 ± 8
7	2.3 ± 0.1	Ketanserin	2500	15.1 ± 1.3	85 ± 8	−24 ± 5*	267 ± 9	−21 ± 11*

[a] Absolute changes in MAP and HR were determined between time 30 min and base line.
[b] ND, not determined.
* P < .05 vs. vehicle-treated group.

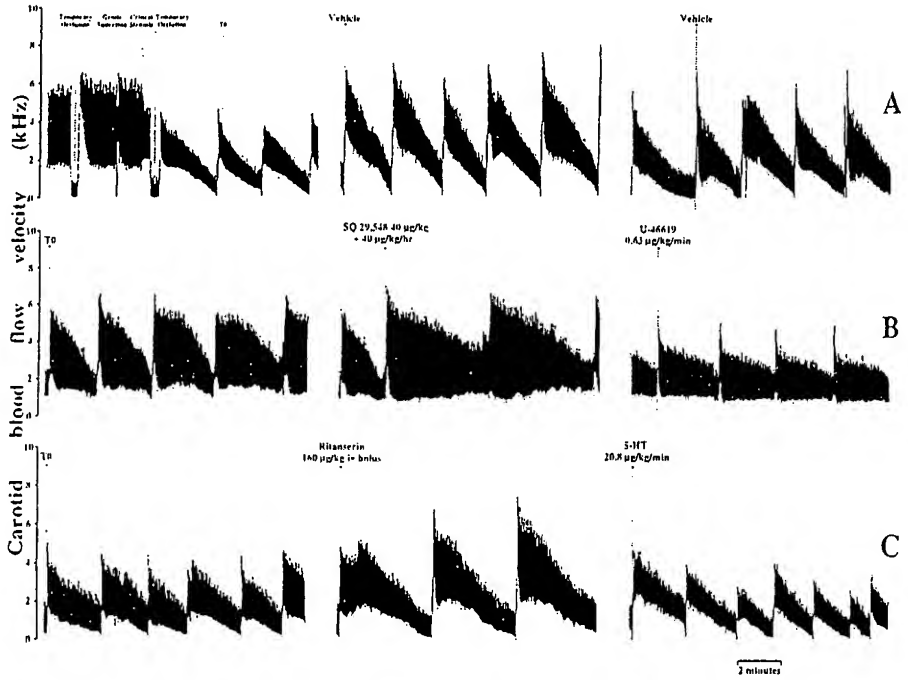

Fig. 2. Typical recordings of carotid blood flow velocity measured with a pulsed Doppler flow probe. A segment of the carotid artery was deendothelialized by gentle squeezing of the artery between a pair of forceps. An external silicone cylinder was then placed around it, and critical stenosis was achieved by graded inflation of an angioplasty balloon placed between the cylinder and the carotid artery. Critical stenosis was confirmed by abolition of hyperemia seen after a temporary (20-sec) complete occlusion of the carotid artery. Critical stenosis at the site of endothelial injury led to the development of gradual reductions of blood flow, followed by either spontaneous or induced (by gentle shaking of the cylinder) restorations of flow to base-line levels (*i.e.*, postcritical stenosis). The figure illustrates typical responses to i.v. administration of either the vehicle (2 mM Na_2CO_3) (A), SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) followed by local (*i.e.*, through the cranial thyroid artery) infusion of the TxA_2 analog U-46619 (0.63 μg/kg/min) (B) or ritanserin (160 μg/kg bolus) followed by local infusion of 5-HT (20.8 μg/kg/min) (C). T0 was considered as the beginning of CFRs.

μg/kg SQ 29,548. The highest dose abolished CFRs in seven of eight rabbits at the end of the 30-min observation period and produced a maximal CFR frequency reduction of 80 ± 8% (P < .05 *vs.* base line). Reduction of CFR frequency by SQ 29,548 occurred without significant changes in MAP or HR, compared with vehicle-infused animals. Furthermore, in an additional group of six rabbits to which SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) was administered, infusion of U-46619 through the cranial thyroid artery, at the dose of 0.63 μg/kg/min, restored CFR frequency to vehicle group levels (from 22.7 ± 3.0 to 9.0 ± 3.3 and then 18.0 ± 6.6 cycles/hr, at base line, first and second period, respectively of the U-46619 infused group; P = NS for second period *vs.* base line and vehicle group; P < .05 for second period *vs.* first period; Fig. 3A). Interestingly, U-46619 failed to restore CFRs in two rabbits whose CFRs had been abolished by SQ 29,548.

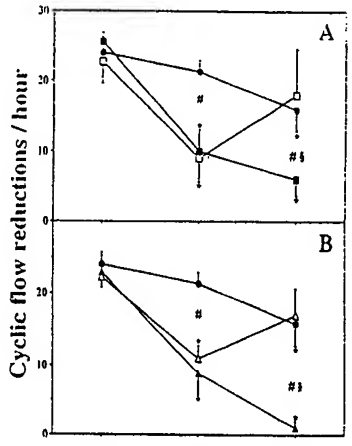

Fig. 3. Influence of pharmacological activation of TP or $5\text{-HT}_{2A/C}$ receptors on CFR frequency of rabbits pretreated with SQ 29,548 (A) or ritanserin (B), respectively. A, rabbits received either the vehicle (●) or SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) alone (n = 10) (■) or followed by local infusion of U-46619 (0.63 μg/kg/min; n = 6) (□). B, animals received either the vehicle (●) or ritanserin (160 μg/kg) alone (n = 11) (▲) or followed by local infusion of 5-HT (20.8 μg/kg/min) (△). Values are mean ± S.E.M. *P < .05 *vs.* base line; #P < .05 for SQ 29,548 or ritanserin alone *vs.* vehicle-infused groups; §P < .05 for SQ 29,548 or ritanserin alone or followed by U-46619 or 5-HT, respectively. CFRs initiated at −15 min were stabilized for 15 min (base line), followed by two consecutive 30-min periods. U-46619, 5-HT or vehicle was perfused i.v. over the 30- to 60-min period.

Influence of ketanserin and ritanserin on CFR frequency. Results are presented in table 3 and figures 2 and 3. Administration of either ketanserin or ritanserin, 15 min after initiation of CFRs, dose-dependently reduced CFR frequency over the first 30 min of observation, with significant inhibition from 40 and 20 μg/kg, respectively (both P < .05), giving ID_{50} values of 89 μg/kg (95% confidence limits, 36–286 μg/kg) and 77 μg/kg (95% confidence limits, 40–132 μg/kg), respectively. The highest doses of ketanserin and ritanserin (2500 μg/kg) abolished CFRs in four of five and six of six rabbits, respectively, and produced maximal reductions in CFR frequency of 83 ± 7% and 98 ± 2% (both P < .05 *vs.* base line). MAP was significantly reduced by the high dose of ketanserin (ΔMAP = −21 ± 7 mm Hg; P < .05 *vs.* vehicle group), whereas no significant reduction was observed in ritanserin-treated, compared with vehicle-treated, animals. HR was statistically significantly reduced by the high dose (2500 μg/kg) of ketanserin and ritanserin (ΔHR = −36 ± 22 and −36 ± 9 beats/min respectively; P < .05 *vs.* vehicle group). In additional experiments, local infusion of exogenous 5-HT (20.8 μg/kg/min, n = 11) in rabbits pretreated with 160 μg/kg ritanserin restored CFR frequency to vehicle group levels (from 22.2 ± 1.4 to 10.9 ± 2.0 and then 17.1 ± 3.7 cycles/hr, at base line, first and second period, respectively, of the 5-HT infused group; P = NS for second period *vs.* base line and vehicle group; P < .05 for second period *vs.* first period; Fig. 3B). 5-HT was also unable to restore CFRs in three rabbits whose CFRs had been abolished by ritanserin.

Influence of $5\text{-HT}_{1B/D}$ and *alpha*-1 adrenergic receptor blockade and COX inhibition on CFR frequency. Because both ketanserin and ritanserin have affinity for $5\text{-HT}_{1B/D}$ receptors, we addressed the possibility that inhibitory activities of both compounds on CFR frequency could be mediated through $5\text{-HT}_{1B/D}$ receptor blockade. For this purpose, we determined whether CFR frequency could be reduced by the novel and highly selective $5\text{-HT}_{1B/D}$ receptor antagonist GR 127935. Administration of GR 127935 (630 μg/kg i.v.) did not statistically significantly reduce CFR frequency or modify MAP or HR, compared with vehicle-infused animals (table 3).

To further evaluate whether the activity of ketanserin on CFR frequency could be related to its *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive effects, we explored whether CFR frequency could be reduced by the *alpha*-1 adrenoceptor antagonist prazosin, at a dose (160 μg/kg i.v.) producing systemic hypotension equivalent to that induced by the highest dose of ketanserin studied (ΔMAP = −29 ± 5 *vs.* −21 ± 7 mm Hg; both P < .05 *vs.* vehicle-treated rabbits and P = NS between groups). Under these conditions, prazosin did not statistically significantly reduce CFR frequency, with respect to vehicle-treated animals (table 3).

Finally, to verify the platelet dependency of CFRs under our experimental conditions, we determined whether CFR frequency could be reduced by the COX inhibitor aspirin. Acute i.v. administration of aspirin, 15 min after initiation of CFRs, dose-dependently reduced CFR frequency over the first 30 min of observation, by 59 ± 21 and 92 ± 3% at 2,500 and 10,000 μg/kg, respectively, without statistically significantly affecting MAP or HR (table 3).

Discussion

The present studies performed in anesthetized rabbits demonstrated that the TP receptor antagonist SQ 29,548 dose-dependently inhibited thrombus formation in both low- and high-shear rate arterial thrombosis, whereas ketanserin and ritanserin were effective only in the high-shear rate model of CFRs. The damping activity of ketanserin and ritanserin on CFR frequency could not be attributed to $5\text{-HT}_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive activities but, rather, was attributed to $5\text{-HT}_{2A/C}$ receptor antagonist properties. Furthermore, local infusions of either the TxA_2 analog U-46619 or 5-HT to animals pretreated with SQ 29,548 or ritanserin, respectively, restored CFR frequency to vehicle-infused levels. These results strongly suggest that TP receptors are involved in arterial thrombosis in rabbits independently of the shear rate, whereas $5\text{-HT}_{2A/C}$ receptors play a major role only in high-shear rate thrombus formation.

TABLE 3

Influence of drugs on CFR frequency and hemodynamic parameters

Values are mean ± S.E.M. SQ 29,548, GR 127935 and prazosin were used to block TP, 5-$HT_{1B/D}$ and *alpha*-1 adrenoceptors, respectively. ID_{50} refers to the geometric mean antagonist dose (with 95% confidence intervals (CI) in parentheses) inhibiting responses by 50%. Absolute changes in MAP and HR were determined between time 30 min and base line.

Number	Body Weight	Treatment	Dose	CFR	ID_{50} (95% CI)	MAP		HR	
						Base line	Absolute Changes	Base line	Absolute Changes
	kg		μg/kg	% inhibition	μg/kg	mm Hg		beats/min	
15	2.6 ± 0.1	Vehicle	1 ml/kg	10 ± 3		89 ± 3	−8 ± 3	285 ± 7	−3 ± 5
6	3.0 ± 0.1	SQ 29,548	10	10 ± 5	35 (24–58)	76 ± 4	−10 ± 4	270 ± 17	2 ± 11
6	2.7 ± 0.1	SQ 29,548	20	28 ± 14		83 ± 7	−15 ± 4	269 ± 20	−20 ± 10
10	2.6 ± 0.0	SQ 29,548	40	61 ± 12*		73 ± 5	−4 ± 4	249 ± 8	−3 ± 7
8	2.6 ± 0.1	SQ 29,548	160	80 ± 8*		66 ± 6	−1 ± 4	272 ± 9	−3 ± 5
4	2.8 ± 0.1	Ritanserin	10	16 ± 4	77 (40–132)	74 ± 10	−5 ± 3	253 ± 27	−25 ± 10
6	2.7 ± 0.0	Ritanserin	20	37 ± 15*		83 ± 5	−15 ± 3	280 ± 18	−7 ± 9
6	2.8 ± 0.0	Ritanserin	40	38 ± 7*		77 ± 5	−3 ± 2	260 ± 8	−7 ± 4
13	2.6 ± 0.1	Ritanserin	160	59 ± 9*		66 ± 8	−12 ± 3	270 ± 10	−4 ± 5
5	2.4 ± 0.2	Ritanserin	630	83 ± 8*		69 ± 10	−2 ± 10	251 ± 30	−18 ± 9
6	2.4 ± 0.1	Ritanserin	2,500	98 ± 2*		80 ± 5	−9 ± 4	271 ± 14	−36 ± 9*
4	2.8 ± 0.1	Ketanserin	10	14 ± 5	89 (36–285)	88 ± 11	−3 ± 6	239 ± 14	−15 ± 14
5	2.5 ± 0.1	Ketanserin	40	48 ± 15*		74 ± 2	−1 ± 5	254 ± 17	−15 ± 5
5	2.8 ± 0.0	Ketanserin	160	56 ± 14*		93 ± 10	−9 ± 3	273 ± 13	−18 ± 7
6	2.4 ± 0.1	Ketanserin	630	74 ± 16*		100 ± 9	−8 ± 5	277 ± 13	−1 ± 10
5	2.4 ± 0.1	Ketanserin	2,500	83 ± 7*		86 ± 5	−21 ± 7*	279 ± 16	−36 ± 22*
9	2.7 ± 0.0	GR 127935	630	21 ± 6		65 ± 5	3 ± 2	233 ± 11	−6 ± 5
5	2.3 ± 0.1	Prazosin	160	29 ± 14		82 ± 4	−29 ± 5*	272 ± 8	−8 ± 7
3	2.9 ± 0.1	Aspirin	2,500	59 ± 21*	<2500	102 ± 1	−12 ± 4	291 ± 11	9 ± 9
5	2.1 ± 0.0	Aspirin	10,000	92 ± 3*		88 ± 4	−14 ± 4	251 ± 10	−2 ± 9

* $P < .05$ vs. vehicle-treated group.

Involvement of TP and 5-$HT_{2A/C}$ receptors in high-shear rate arterial thrombosis. In the carotid stenosis and endothelial injury model, SQ 29,548, ritanserin and ketanserin all dose-dependently reduced CFR frequency (ID_{50} values of 35, 77 and 89 μg/kg, respectively). The inhibitory activity of ketanserin and ritanserin on CFRs could not be attributed to either 5-$HT_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive activities (see below) but, rather, was attributed to 5-$HT_{2A/C}$ receptor antagonist properties. Local infusion of U-46619, a stable TxA_2 analog, or 5-HT at the site of the injury restored CFR frequency to vehicle-infused levels in animals whose CFRs had been reduced, but not abolished, by SQ 29,548 or ritanserin, respectively. These results provided further evidence that TP and 5-$HT_{2A/C}$ receptors mediated thrombus formation and maintenance in the CFR experiments, in agreement with previous reports (Willerson *et al.*, 1989; Golino *et al.*, 1990, 1992, 1993; Torr *et al.*, 1990; Salvati *et al.*, 1993; Beaughard *et al.*, 1995). Furthermore, aspirin also dose-dependently reduced CFR frequency, thus confirming the platelet-dependent CFR occurrence described in dogs with coronary artery stenosis and endothelial injury (Folts, 1995).

In addition to possessing nanomolar affinity for 5-$HT_{2A/C}$ receptors (for reviews, see Zifa and Fillion, 1992; Hoyer *et al.*, 1994), ketanserin and ritanserin also have affinity for 5-$HT_{1B/D}$ receptors (Weinshank *et al.*, 1991; Pauwels *et al.*, 1995). Therefore, we addressed the possibility that inhibitory activities of ketanserin and ritanserin on CFR frequency could be mediated through 5-$HT_{1B/D}$ receptor antagonism. The novel and highly selective receptor antagonist GR 127935 (Clitherow *et al.*, 1994; Skingle *et al.*, 1994), at a dose (630 μg/kg i.v.) that is higher than that required to fully block 5-$HT_{1B/D}$ receptor-mediated carotid vasoconstriction in anesthetized pigs (De Vries *et al.*, 1996), did not alter CFR frequency or hemodynamic parameters. Thus, these results provide evidence that 5-$HT_{1B/D}$ receptors are apparently not involved in mediating thrombus formation under the present experimental conditions.

The damping activity of ketanserin on CFR frequency cannot be related to *alpha*-1 adrenoceptor antagonist properties (Leysen *et al.*, 1981) either, because the selective *alpha*-1 adrenoceptor antagonist prazosin, at a dose producing systemic hypotension equivalent to that induced by the highest dose of ketanserin (2,500 μg/kg), did not reduce CFR frequency. These results suggest that *alpha*-1 adrenoceptors are not involved in mediating arterial thrombus formation under high-shear rate conditions.

Both TxA_2 and 5-HT are implicated in the pathogenesis of platelet aggregation and dynamic vasoconstriction that occur at sites of endothelial injury and coronary artery stenosis. When platelets aggregate, they release (among other factors) 5-HT, which causes local vasoconstriction and acts to amplify and further promote aggregation. It might be expected that vasodilation (reflected by systemic hypotension), as observed after administration of the highest dose of ketanserin investigated, would counteract the local vasoconstriction induced by the vasoactive agents released during aggregation and would thus reduce further aggregation (*i.e.*, reduce CFR frequency). This possibility can be excluded because 1) a statistically significant reduction in CFR frequency was observed at doses of ketanserin that did not affect MAP (≤630 μg/kg), 2) ritanserin at a dose that reduced CFR frequency to the same extent as that produced by the highest dose of ketanserin (83 ± 8% at 630 μg/kg *vs.* 83 ± 7% at 2,500 μg/kg) did not reduce MAP and, 3) at a dose producing systemic hypotension equivalent to that evoked by the highest dose of ketanserin, the *alpha*-1 adrenoceptor antagonist prazosin did not significantly reduce CFR frequency. Moreover both ketanserin and ritanserin, at the highest dose investigated (2,500 μg/kg), induced bradycardia, which could reduce cardiac output, as reported by Bolt and Saxena (1985), and possibly carotid blood flow. In fact, ritanserin at a dose that reduced CFR frequency to the same extent as that produced by the highest dose of ketanserin (83 ± 8% at 630 μg/kg *vs.* 83 ± 7% at 2,500 μg/kg) did not alter HR, thus excluding bradycardia as a major antithrombotic mechanism of action of these drugs. Taken together, these results provide evidence that the 5-$HT_{1B/D}$ and *alpha*-1 adrenergic antagonist properties of ketanserin and ritanserin are not involved in reducing CFR frequency, thus confirming that both 5-$HT_{2A/C}$ and TP receptor activation are major mechanisms involved in CFR occurrence and maintenance in stenotic and endothelially injured rabbit carotid arteries.

Differential involvement of TP and 5-$HT_{2A/C}$ receptors in low-shear rate arterial thrombosis. In contrast to ketanserin and ritanserin, SQ 29,548 significantly and dose-dependently inhibited arteriovenous shunt occlusion without affecting hemodynamic parameters. Inactivity of ketanserin and ritanserin in the present experimental model cannot be explained by the use of inadequately low doses, because 2,500 μg/kg is relatively high, compared with doses that substantially inhibit 5-$HT_{2A/C}$ receptor-mediated responses *in vivo* (Bolt and Saxena, 1985; Pettersson *et al.*, 1985; Docherty, 1989; Valentin *et al.*, 1995). Antithrombotic inactivity of ketanserin has previously been reported in an arteriovenous shunt model in rats (Maffrand *et al.*, 1988). In addition, such doses of ketanserin and ritanserin are likely to have extensively blocked *alpha*-1 adrenergic (Bolt and Saxena, 1985; Pettersson *et al.*, 1985; Docherty, 1989) or 5-$HT_{1B/D}$ (De Vries *et al.*, 1996) receptors, respectively (see above), thereby excluding any involvement of *alpha*-1 adrenoceptors or 5-$HT_{1B/D}$ receptors in arterial thrombus formation under low-shear rate conditions. 5-$HT_{2A/C}$ and TP receptors clearly do not share similar involvement in mediating arterial thrombus formation under low-shear rate conditions. Moreover, the antithrombotic effectiveness of SQ 29,548 can be accounted for by inhibition of locally produced TxA_2/endoperoxides, which elicit platelet aggregation (Ogletree, 1987). The extracorporeal arteriovenous shunt was previously described in rats as a platelet-predominant thrombosis model (Umetsu and Sanai, 1978; Shand *et al.*, 1984), and the histological analyses of thrombus composition we performed (data not shown) confirm and extend these observations to rabbits. Evidence is therefore presented that platelet activation is a key component of arterial thrombus formation under low-shear rate conditions in the rabbit arteriovenous shunt. Interestingly, no information is currently available on the existence of putative platelet 5-$HT_{1B/D}$ receptors. Thus, platelet activation is mediated partly through TP receptor stimulation, whereas platelet 5-$HT_{2A/C}$ receptors appear to have little or no involvement.

Thrombus formation in high- *vs.* low-shear rate arterial thrombosis. A major finding of the present study was that SQ 29,548 elicited antithrombotic activity independently of the shear rate, whereas ketanserin and ritanserin exerted substantial antithrombotic activity only when shear rates were high. High shear rates, such as those found in the present study, are not physiological (20,000–60,000 sec^{-1}) but are reached under pathological conditions in stenotic arteries (Goldsmith and Turitto, 1986; Strony *et al.*, 1993). The way in which shear stress can induce aggregation of platelets is gradually being elucidated. It is now established that the GP IIb/IIIa receptor, the final common pathway of platelet aggregation, interacts only with fibrinogen in a low-shear rate environment, whereas it interacts mainly with vWf in a high-shear rate environment (Ruggeri, 1994). The role of vWf appears to be most significant at high shear rates, presumably as a consequence of its unique molecular architecture. Under the effects of high shear forces, vWf molecules take the shape of extended filaments; the repeating subunit structure of these large multimers offers an array of interaction sites capable of binding in a multivalent manner to receptors on the platelet membrane, thereby increasing the number of contact points and the strength of interaction. As a result, the overall force linking platelets to the surface and/or to one another is increased. This interpretation of events explains why the role of vWf is less relevant at lower shear rates, because other adhesive molecules may provide sufficient force of interaction to withstand opposing shear forces of lesser magnitude (Chow *et al.*, 1992; Ikeda *et al.*, 1993; Ruggeri, 1994). In remarkable contrast, GP IIb/IIIa in a low-shear rate environment shows the ability to interact only with immobilized fibrinogen (Savage and Ruggeri, 1991). Interestingly, monoclonal antibodies directed against GP IIb/IIIa and GP IIb/IIIa receptor antagonists have demonstrated high efficacy in situations of both low (Ikeda *et al.*, 1991) and high (Coller and Scudder, 1985; Gold *et al.*, 1988; Shebuski *et al.*, 1989a,b; Chow *et al.*, 1992) shear rates, confirming the pivotal role of GP IIb/IIIa in the process of thrombus formation, independently of shear rate. Furthermore, Golino *et al.* (1995) recently demonstrated the key role of GP IIb/IIIa in the stenotic and endothelially injured rabbit carotid artery model (Golino *et al.*, 1995).

Differential antithrombotic effectiveness of ketanserin and ritanserin, even at relatively high doses (Valentin *et al.*, 1995), is also in agreement with different mechanisms mediating thrombus formation at high *vs.* low shear rates and strongly suggests that 5-HT plays a major role in thrombus growth only under high-shear rate conditions. A role for 5-HT in mediating the formation of arterial thrombi under low-shear rate conditions cannot, however, be excluded, because the indoleamine has been reported to mediate platelet aggregation *in vitro*, albeit weakly, and to contribute to aggregate growth *in vivo* (Menys, 1993). The basis for a major role of 5-HT in high-shear rate thrombus formation, compared with a minor role with low shear rates, is unclear at present but could involve enhanced 5-HT release from platelet dense granules with high shear rates. It is well established that, *in vitro*, high shear stresses (>50 dyn/cm^2) activate spontaneous or agonist-induced aggregation by the release of platelet dense granule contents (Brown *et al.*, 1975). This would be compatible with the increased transcardine 5-HT concentrations that have been observed in patients with coronary stenoses (Van den Berg *et al.*, 1989). Factors other than shear rate may have influenced the differential responsiveness of 5-$HT_{2A/C}$ receptor antagonists in the two models. Differences in 1) thrombogenic substrate between the two models (i.e., silk thread *vs.* exposed subendothelial collagen) and 2) the size of the thrombogenic surface may also be involved in the differential antithrombotic effectiveness of ketanserin and ritanserin, which would also lend support to the hypothesis

that the mechanisms of thrombus formation, in particular platelet activation, are different under high- *vs.* low-shear rate conditions. In contrast to the 5-$HT_{2A/C}$ receptor, TP receptor activation plays a major role in both high- and low-shear rate arterial thrombosis. A possible explanation is that arachidonic acid metabolism in platelet membranes, leading to TxA_2/endoperoxide production and consequent TP receptor activation, occurs independently of the mechanism of platelet activation *in vivo* (Badimon *et al.*, 1992; Reilly and FitzGerald, 1993). Although 5-$HT_{2A/C}$ and TP receptor antagonists have demonstrated efficacy in preventing thrombus formation in high-shear rate situations, the relative physiological and pathological importance of these mechanisms is probably moderate, compared with functional antagonism of GP IIb/IIIa.

In conclusion, our results indicate that TP receptors are involved in arterial thrombosis in rabbits, independently of the shear rate, whereas 5-$HT_{2A/C}$ receptors play a role only in high-shear rate thrombus formation. The precise mechanism underlying the differential role of 5-$HT_{2A/C}$ receptors in high- *vs.* low-shear rate arterial thrombosis deserves further study.

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Differential Involvement of Serotonin 2A/C and Thromboxane A_2/Prostanoid Receptors in High- *vs.* Low-Shear Rate Arterial Thrombosis in Rabbits[1]

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ABSTRACT

Experiments performed in 226 pentobarbitone-anesthetized rabbits were designed to investigate the involvement of thromboxane/prostanoid and 5-hydroxytryptamine (5-HT)$_{2A/C}$ receptors during arterial thrombus formation in distinct low- and high-shear rate thrombosis models. Antithrombotic activities of the thromboxane/prostanoid receptor antagonist SQ 29,548 and two chemically distinct 5-HT$_{2A/C}$ receptor antagonists, ritanserin and ketanserin, were assessed first in low-shear rate (~600 sec^{-1}) arterial thrombosis, produced by insertion of a silk thread as thrombogenic substrate into the central section of an extracorporeal arteriovenous shunt established between the left carotid artery and the right jugular vein (n = 77), and second in high-shear rate (~40,000 sec^{-1}) arterial thrombosis, produced by critical stenosis and local endothelial injury of a carotid artery, characterized by cyclic flow reductions (CFRs) due to recurrent platelet aggregation and subsequent dislodgement of the thrombus (n = 149). Under low shear rate, SQ 29,548 (10–2500 μg/kg plus 10–2500 μg/kg/hr i.v.), but not ritanserin or ketanserin (both at 2500 μg/kg i.v.), dose-dependently inhibited thrombus formation. In contrast, under high shear rate, SQ 29,548 (10–160 μg/kg plus 10–160 μg/kg/hr i.v.) and both ritanserin and ketanserin (both at 10–2500 μg/kg i.v.) dose-dependently reduced CFR frequency, with ID_{50} values of 35 μg/kg (95% confidence limits, 24–58 μg/kg), 77 μg/kg (95% confidence limits, 40–132 μg/kg) and 89 μg/kg (95% confidence limits, 36–285 μg/kg) i.v., respectively. Furthermore, local infusion of the stable thromboxane A_2 analog U-46619 (0.63 μg/kg/min) or 5-HT (20.8 μg/kg/min) proximal to the site of injury and stenosis in rabbits pretreated with either SQ 29,548 (40 μg/kg plus 40 μg/kg/hr i.v.) or ritanserin (160 μg/kg i.v.), respectively, restored CFR frequency to vehicle group levels in animals whose CFR frequency was previously reduced. The inhibitory activity of ketanserin and ritanserin on CFRs could not be attributed to 5-HT$_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties or to any hypotensive activity. These results provide firm evidence that thromboxane/prostanoid receptors are involved in arterial thrombosis in rabbits independently of the shear rate, whereas 5-HT$_{2A/C}$ receptors play a major role only in high-shear rate thrombus formation.

Platelet aggregation is influenced by shear forces (Ruggeri, 1994). In particular, GP IIb/IIIa, the final common pathway of platelet aggregation, interacts only with fibrinogen in a low-shear rate environment, whereas it interacts mainly with vWf in a high-shear rate environment (Ruggeri, 1994). Platelet activation plays an important role in arterial thrombosis (Badimon *et al.*, 1992) because, early in the formation of the hemostatic plug, platelet aggregates are formed at the site of vessel injury, bifurcations or stenoses, which present local increases in shear rates (Goldsmith and Turitto, 1986; Strony *et al.*, 1993). To mimic pathophysiological conditions, Folts developed a model of CFRs in critically stenotic canine coronary artery with endothelial damage, thereby producing high-shear rate thrombosis (Folts *et al.*, 1976; Folts, 1995). Ashton *et al.* (1987, 1989) and Golino *et al.* (1989, 1990) showed that both TxA_2 and 5-HT mediated CFRs in this canine model, *via* 5-HT$_2$ and TP receptor activation, respectively. Furthermore, elevated blood levels and tissue concentrations of TxA_2 and 5-HT have been detected around the stenosis and in the distal canine coronary arterial blood (Schmitz *et al.*, 1985; Ashton *et al.*, 1986). However, the roles of 5-HT$_{2A/C}$ and TP receptors in promoting thrombosis under low-shear rate situations are less well documented (Muffrand *et al.*, 1988; Ruggeri, 1994).

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ABBREVIATIONS: CFR, cyclic flow reduction; COX, cyclooxygenase; GP, glycoprotein; HR, heart rate; 5-HT, 5-hydroxytryptamine; I.D., internal diameter; MAP, mean arterial pressure; NS, not significant; SQ 29,548, [1S-[1α,2α(5Z),3α,4α]]-7-[3-[[2-(phenylaminocarbonyl)hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; TP receptor, thromboxane A_2/prostanoid receptor; TxA_2, thromboxane A_2; U-46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$; vWf, von Willebrand factor.

The aim of the present study was to investigate further the involvement of TP and 5-$HT_{2A/C}$ receptors during arterial thrombus formation in distinct low- and high-shear rate thrombosis models in anesthetized rabbits. 1) in an extracorporeal arteriovenous shunt model and 2) in arterial thrombosis produced by critical stenosis and local endothelial injury of a carotid artery. To do so, we used the selective and neutral TP receptor antagonist SQ 29,548 (Ogletree *et al.*, 1985; Bertolino *et al.*, 1995) and the nonselective 5-$HT_{2A/C}$ receptor antagonists ritanserin and ketanserin (Baxter *et al.*, 1995). Because both ketanserin and ritanserin also have affinities for *alpha*-1 adrenoceptors (Leysen *et al.*, 1981, 1985) and/or 5-$HT_{1B/D}$ receptors (Weinshank *et al.*, 1991; Pauwels *et al.*, 1995), the role of these receptors was investigated using the *alpha*-1 adrenoceptor antagonist prazosin and the novel and highly selective 5-$HT_{1B/D}$ receptor antagonist GR 127935 (Clitherow *et al.*, 1994; Skingle *et al.*, 1994). The nonselective COX inhibitor aspirin was also studied.

Materials and Methods

General procedure. Experiments were carried out in accordance with French law and local ethics committee guidelines for animal research. Animals were housed in climate-controlled conditions (21°C and 55% relative humidity, with a 12-hr light/dark cycle) and provided standard chow and water *ad libitum*. On the day of the experiment, male New-Zealand White rabbits (2.2–3.1 kg; Elevage Scientifique, Des Dombes, Chatillon Sur Chalaronne, France) were anesthetized with an injection of sodium pentobarbital (30 mg/kg; Sanofi Laboratories, Libourne, France), administered through the marginal ear vein, and were then placed on a table under an homeothermic blanket to maintain rectal temperature at 39.5 ± 0.5°C. Through a median incision of the neck, animals underwent tracheotomy and were mechanically ventilated (Harvard Apparatus, South Natick, MA). Polyethylene catheters (I.D., 0.58 mm; Biotrol-Merck, Paris, France) were inserted into a femoral artery and vein for respectively, infusing fluids and drugs, sampling blood and continuously measuring arterial pressure *via* a Statham P10EZ pressure transducer (Viggo-Spectramed, Oxnard, CA) connected to a Gould amplifier (model 13–4615-50; Gould Instruments, Longjumeau, France). The analog arterial pressure signal was digitized (model MP 100; Biopac Systems, Goleta, CA) and simultaneously recorded by means of data acquisition software (AcqKnowledge 881 version 3.1.1; Biopac Systems).

Extracorporeal arteriovenous shunt model (low shear rate). Animals were prepared according to the method described for rats by Umetsu and Sanai (1978) and Shand *et al.* (1984) and modified by Freund *et al.* (1993). Briefly, the right jugular vein and left carotid artery were exposed and carefully isolated from surrounding tissues. The shunt (30 cm in length) was constructed with polyethylene catheters (Biotrol-Merck, France) as follows: the sections, which were inserted into a rabbit carotid artery and jugular vein, consisted of 12.5-cm-long catheters (I.D., 1.14 mm). They were connected to the central part of the shunt *via* a 6-cm-long catheter (I.D., 2 mm). A silk thread (Gutermann Laska, Paris, France), placed in the central portion of the shunt, was used as the thrombogenic substrate when exposed to the circulation by unclamping of the shunt. The polyethylene tubing used was coated with silicone (Silisonde, Vygon, Ecouen, France). The shunt was filled with a 0.1 ml/kg heparin solution (50 IU/ml; Choay Laboratories, Paris, France). After clamping, one extremity of the shunt was inserted into the left carotid artery and then the other was inserted into the right jugular vein. A thermal microprobe (type IT-23; Physitemp Instruments Inc., Clifton, NJ) was secured onto the central part of the shunt. Blood flow was then established through the shunt by unclamping, thereby rapidly raising the shunt temperature to values slightly lower than the rectal temperature of the animal (fig. 1). Shunt temperature reached a plateau and then fell rapidly, coinciding with increasing thrombotic obstruction of blood flow across the shunt. Occlusion time of the shunt was defined as the elapsed time between the start of blood flow and the time at which the shunt temperature was 1°C higher than the base-line temperature (*i.e.*, before blood flow start) and corresponded to the formation of an occlusive thrombus and complete interruption of blood flow (Freund *et al.*, 1993).

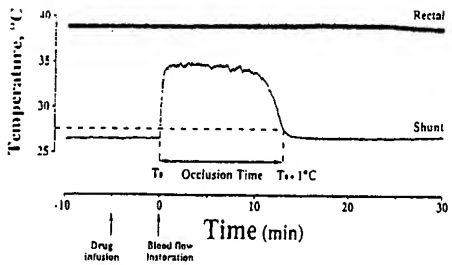

Fig. 1. Typical recordings of rectal and shunt temperatures in an arteriovenous shunt experiment in rabbits and the determination of shunt occlusion time, defined as the elapsed time between T0 and T0 + 1°C. T0 refers to base-line temperature reached by the shunt during complete occlusion.

Influence of drugs on shunt occlusion. Five minutes before shunt blood flow was established, animals received an i.v. injection of either 1) vehicle (0.9% NaCl, n = 5; 0.9% NaCl plus ethyl alcohol, 9:1, v/v, n = 6; or 2 mM Na_2CO_3, n = 16), 2) SQ 29,548 (10, 40, 160, 630 or 2500 μg/kg, n = 4–8 rabbits/group) administered over 2 min as a 1 ml/kg solution, followed by a constant infusion of 10 to 2500 μg/kg/hr (rate, 40 μl/min), 3) ketanserin (n = 7 rabbits) or 4) ritanserin (n = 8 rabbits).

Carotid stenosis and endothelial injury model (high shear rate). Animals were prepared according to a modification of the method described by Golino *et al.* (1992). A common carotid artery was exposed and carefully isolated from the surrounding tissues. The cranial thyroid artery, a small carotid artery collateral, was cautiously exposed and a polyethylene catheter was inserted until the ostium of the carotid artery was reached, thus allowing local infusion of drugs and vehicles. Saline was continuously infused through the catheter at the rate of 40 μl/min, to maintain patency. Carotid blood flow velocity was continuously measured with a pulsed Doppler flow probe (20 MHz, model HVPD-20; Crystal Biotech, Hopkinton, MA) placed proximally to the cranial thyroid artery. Thereafter, a segment of the exposed carotid artery, distal to the cranial thyroid artery, was deendothelialized by gentle squeezing of the artery between a pair of forceps. An external silicone cylinder (7-mm wide; I.D., 3 mm) was placed around it, and critical stenosis was achieved by graded inflation of an angioplasty balloon (model 3F; Solo, USCI-Bard Laboratories, Paris, France) placed between the cylinder and the carotid artery. Critical stenosis was confirmed by the absence of hyperemia after a temporary (20-sec) complete occlusion of the carotid artery (fig. 1). Once induced, CFRs were observed for 15 min (base line) and for two consecutive 30-min periods. CFR frequencies were quantified for each period and expressed per hour.

Influence of drugs on CFR frequency. After 15 min of CFRs, animals received either 1) vehicle (0.9% NaCl, n = 6; 0.9% NaCl plus ethyl alcohol, 9:1, v/v, n = 4; or 2 mM Na_2CO_3, n = 5), 2) SQ 29,548 (10, 20, 40 or 160 μg/kg; n = 6–10 rabbits/group) administered over 2 min as a 1 ml/kg solution, followed by a constant infusion of 10 to 160 μg/kg/hr, 3) ritanserin (10, 20, 40, 160, 630 or 2500 μg/kg, n = 5–13 rabbits/group) or 4) ketanserin (10, 40, 160, 630 or 2500 μg/kg, n = 4–6 rabbits/group). In two additional groups of rabbits the effects of local infusion, through the cranial thyroid artery, of exogenous 5-HT (20.8 μg/kg/min, n = 11) or the stable TxA_2 analog U-46619 (0.63 μg/kg/min, n = 6) on CFR frequency were assessed in rabbits pretreated with 160 μg/kg ritanserin or 40 μg/kg plus 40 μg/kg/hr SQ 29,548. 5-HT and U-46619 were perfused during the second 30-min period at a rate of 40 μl/min. 5-$HT_{1B/D}$ and *alpha*-1 adrenoceptor blockade and nonselective COX inhibition were achieved by i.v. administration of GR 127935 (630 μg/kg over 1 hr at a rate of 40 μl/min, n = 9), prazosin (160 μg/kg, n = 5) or aspirin (2,500 μg/kg, n = 3, and 10,000 μg/kg, n = 5), respectively. After drug administration, hemodynamic variables and carotid blood flow velocity were continuously measured during two consecutive periods of 30 min.

Shear rate estimations and statistical evaluation. In arteriovenous shunt experiments, blood flow was determined in a separate group of six rabbits by two consecutive measurements of blood loss over 5 sec, after sectioning of the central part of the shunt. Shear rates were estimated from the Poiseuille law for laminar flow (Goldsmith and Turitto, 1986; Strony *et al.*, 1993), *i.e.*, $\gamma(R) = 4Q/\pi R^3$, where γ is the shear rate (seconds^{-1}), Q is the flow rate (milliliters per second) and R is the radius of the catheter (centimeters).

In CFR experiments, carotid blood flow (in milliliters per minute) was determined in the vehicle-infused group (n = 15) from the blood velocity signal (kilohertz), according to the following formula (Freed *et al.*, 1979): $Q = 1.25 \times d^2 \times \Delta f$, where Q is the flow rate (milliliters per minute), d is the diameter of the carotid artery (millimeters), estimated to be equal to the diameter of the Doppler probe, and Δf is the Doppler frequency shift (kilohertz). Shear rates were estimated proximally to the endothelial site of injury, and critical stenosis was determined according to the above formula.

Data are expressed as means ± S.E.M. Analysis of variance, with or without repeated measures, followed by Dunnett's test was used to assess significance among and between groups, respectively (StatView; Abacus Concepts Inc., Berkeley, CA). Dose-response curves were fitted, using an operational sigmoid model (Marquardt, 1963), from relative inhibition of CFR determined 30 min after drug administration (first 30-min period) (Origin; Microcal Software Inc., Northampton, MA); ID_{50} refers to the mean geometric antagonist dose (with 95% confidence intervals in parentheses) inhibiting a response by 50%. P = .05 was considered the minimum level of significance.

Drugs and solutions. SQ 29,548 and U-46619 (Cayman Chemical Co., Ann Arbor, MI) were dissolved in Na_2CO_3 (2 mM) and sterile saline (0.9%), respectively, and maintained on ice. Ritanserin and ketanserin tartrate (Research Biochemicals Inc., Natick, MA) were dissolved in ethyl alcohol plus sterile saline (0.9%; 1:9, v/v) and sterile saline (0.9%), respectively. GR 127935 was synthesized by the Division of Medicinal Chemistry IV, Centre de Recherche Pierre Fabre (Castres, France) and dissolved in sterile saline (0.9%). Prazosin hydrochloride and 5-HT creatinine sulfate (Sigma Chemical Co., St. Louis, MO) were dissolved in sterile saline (0.9%), and 5-HT was maintained on ice. Aspirin (acetylsalicylic acid lysine salt; Synthelabo Laboratories, Paris, France) was dissolved in sterile saline (0.9%). Drugs were administered (as 1 ml/kg solutions), in micrograms per kilogram base weight, except when specified otherwise.

Results

Estimation of shear rates. Results are presented in table 1. Estimated shear rates were markedly higher in arterial thrombosis produced by carotid stenosis and endothelial injury, compared with the arteriovenous shunt model (~40,000 sec^{-1} *vs.* ~600 sec^{-1}).

Influence of drugs on shunt occlusion. Results are presented in table 2. In control, vehicle-infused rabbits, because no statistically significant difference was found among occlusion times for the three groups, data were pooled together. In vehicle-infused rabbits, the occlusion time was 13.7 ± 1.3 min. The interruption of blood flow was associated with a slight reduction in MAP and no statistically significant changes in HR [mean maximal absolute changes: ΔMAP = −9 ± 2 mm Hg and ΔHR = 2 ± 4 beats/min; P < .05 and P = NS *vs.* base line, respectively]. The TP receptor antagonist SQ 29,548 significantly and dose-dependently inhibited thrombus formation, attaining a maximal effect of 31.3 ± 7.3 min (P < .05 *vs.* vehicle group) at the highest dose (2,500 μg/kg) without affecting MAP or HR, compared with vehicle-treated animals. In contrast, neither ritanserin nor ketanserin (both at 2,500 μg/kg) inhibited thrombus formation. A statistically significant reduction in MAP and HR was observed in ketanserin-treated but not ritanserin-treated animals, compared with vehicle-infused rabbits.

Carotid stenosis and endothelial injury model. As shown in figure 2, critical stenosis at the site of endothelial injury, achieved by graded inflation of an angioplasty balloon, led to the development of the typical pattern of gradual reductions of blood flow, followed by either spontaneous or induced (by gentle shaking of the cylinder) restorations of flow to base-line levels (*i.e.*, postcritical stenosis). These CFRs are known to be due to recurrent platelet aggregation at the site of the stenosis, followed by embolization of the thrombus. CFRs developed in all animals (n = 149), with a mean frequency of 23.9 ± 0.5 cycles/hr. In control, vehicle-infused rabbits, no significant change in CFR frequency was observed between base line and the first 30-min period of observation, whereas a slight decrease (~ 25%) in CFR frequency was noted between the first and second periods (fig. 3).

Influence of SQ 29,548 on CFR frequency. Results are presented in table 3 and figures 2 and 3. Administration of SQ 29,548, 15 min after initiation of CFRs, dose-dependently reduced the frequency of CFRs over the first 30-min period of observation. The ID_{50}, determined 30 min after drug administration, was 35 μg/kg (95% confidence limits, 24–58 μg/kg). Significant inhibition of CFR frequency was observed with 40

TABLE 1

Comparison of carotid blood flow and estimated shear rates in arteriovenous shunt and CFR models

Values are mean ± S.E.M. or ranges (in parentheses) for estimated shear rates.

	AVS*	CFR		P Value		
		PST	ST	AVS *vs.* PST	AVS *vs.* ST	PST *vs.* ST
Number	6	15				
Body weight (kg)	2.6 ± 0.1	2.78 ± 0.1				
Blood flow (ml/min)	26.7 ± 1.5	24.8 ± 1.2	14.9 ± 1.1	NS	<0.05	<0.05
Shear rate (sec^{-1})	566 (477–637)	527 (316–640)	39,561 (21,619–61,009)	NS	<0.05	<0.05

* AVS, arteriovenous shunt; PST, prestenosis; ST, stenosis.

TABLE 2

Influence of drugs on thrombotic occlusion time and hemodynamic parameters in the arteriovenous shunt model

Values are mean ± S.E.M.

Number	Body Weight	Treatment	Dose	Occlusion Time	MAP		HR	
					Base line	Absolute Changes[a]	Base line	Absolute Changes[a]
	kg		μg/kg	min	mm Hg		beats/min	
27	2.5 ± 0.1	Vehicle	1 ml/kg	13.7 ± 1.3	88 ± 3	−9 ± 2	284 ± 6	2 ± 4
4	2.5 ± 0.1	SQ 29,548	10	15.5 ± 1.8	ND[b]	ND	ND	ND
4	2.5 ± 0.1	SQ 29,548	40	17.6 ± 3.3	90 ± 8	−8 ± 3	297 ± 14	−1 ± 12
8	3.0 ± 0.1	SQ 29,548	160	22.2 ± 2.8*	94 ± 7	−11 ± 3	307 ± 15	−14 ± 4
8	2.8 ± 0.1	SQ 29,548	630	22.5 ± 4.2*	86 ± 3	−7 ± 4	298 ± 14	−10 ± 8
5	2.8 ± 0.2	SQ 29,548	2500	31.3 ± 7.3*	85 ± 6	−15 ± 5	294 ± 22	−2 ± 6
8	2.1 ± 0.1	Ritanserin	2500	14.7 ± 1.6	75 ± 4	−3 ± 3	281 ± 12	−15 ± 8
7	2.3 ± 0.1	Ketanserin	2500	15.1 ± 1.3	85 ± 8	−24 ± 5*	267 ± 9	−21 ± 11*

[a] Absolute changes in MAP and HR were determined between time 30 min and base line.
[b] ND, not determined.
* $P < .05$ vs. vehicle-treated group.

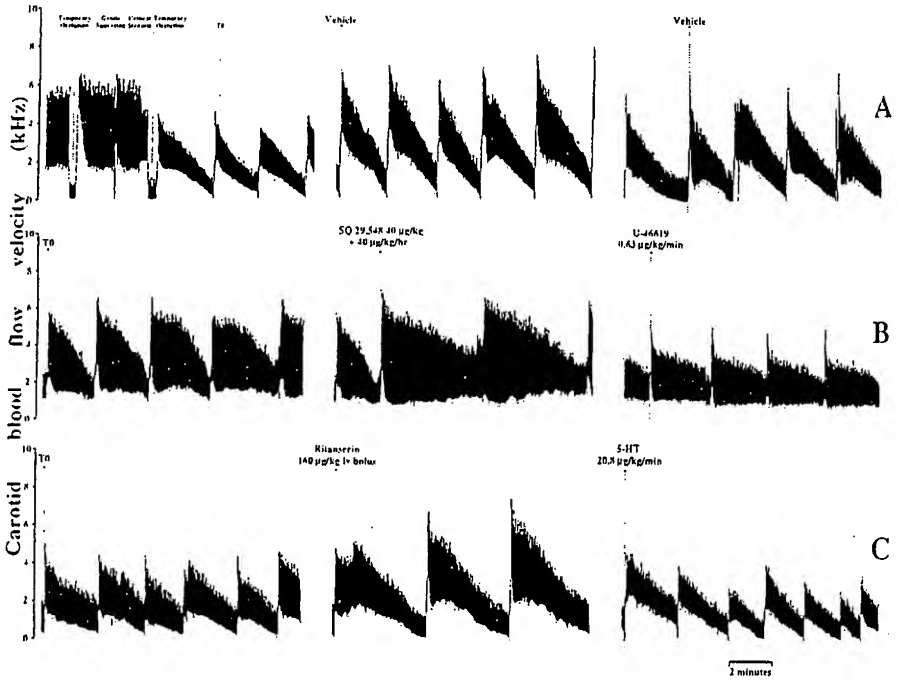

Fig. 2. Typical recordings of carotid blood flow velocity measured with a pulsed Doppler flow probe. A segment of the carotid artery was deendothelialized by gentle squeezing of the artery between a pair of forceps. An external silicone cylinder was then placed around it, and critical stenosis was achieved by graded inflation of an angioplasty balloon placed between the cylinder and the carotid artery. Critical stenosis was confirmed by abolition of hyperemia seen after a temporary (20-sec) complete occlusion of the carotid artery. Critical stenosis at the site of endothelial injury led to the development of gradual reductions of blood flow, followed by either spontaneous or induced (by gentle shaking of the cylinder) restorations of flow to base-line levels (*i.e.*, postcritical stenosis). The figure illustrates typical responses to i.v. administration of either the vehicle (2 mM Na_2CO_3) (A), SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) followed by local (*i.e.*, through the cranial thyroid artery) infusion of the TxA_2 analog U-46619 (0.63 μg/kg/min) (B) or ritanserin (160 μg/kg bolus) followed by local infusion of 5-HT (20.8 μg/kg/min) (C). T0 was considered as the beginning of CFRs.

μg/kg SQ 29,548. The highest dose abolished CFRs in seven of eight rabbits at the end of the 30-min observation period and produced a maximal CFR frequency reduction of 80 ± 8% ($P < .05$ *vs.* base line). Reduction of CFR frequency by SQ 29,548 occurred without significant changes in MAP or HR, compared with vehicle-infused animals. Furthermore, in an additional group of six rabbits to which SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) was administered, infusion of U-46619

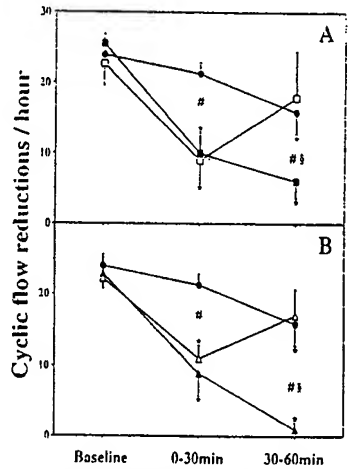

Fig. 3. Influence of pharmacological activation of TP or 5-$HT_{2A/C}$ receptors on CFR frequency of rabbits pretreated with SQ 29,548 (A) or ritanserin (B), respectively. A, rabbits received either the vehicle (●) or SQ 29,548 (40 μg/kg plus 40 μg/kg/hr) alone ($n = 10$) (■) or followed by local infusion of U-46619 (0.63 μg/kg/min; $n = 6$) (□). B, animals received either the vehicle (●) or ritanserin (160 μg/kg) alone ($n = 11$) (▲) or followed by local infusion of 5-HT (20.8 μg/kg/min) (△). Values are mean ± S.E.M. *$P < .05$ *vs.* base line; #$P < .05$ for SQ 29,548 or ritanserin alone *vs.* vehicle-infused groups; §$P < .05$ for SQ 29,548 or ritanserin alone or followed by U-46619 or 5-HT, respectively. CFRs initiated at −15 min were stabilized for 15 min (base line), followed by two consecutive 30-min periods. U-46619, 5-HT or vehicle was perfused i.v. over the 30- to 60-min period.

through the cranial thyroid artery, at the dose of 0.63 μg/kg/min, restored CFR frequency to vehicle group levels (from 22.7 ± 3.0 to 9.0 ± 3.3 and then 18.0 ± 6.6 cycles/hr, at base line, first and second period, respectively of the U-46619 infused group; P = NS for second period *vs.* base line and vehicle group; $P < .05$ for second period *vs.* first period; Fig. 3A). Interestingly, U-46619 failed to restore CFRs in two rabbits whose CFRs had been abolished by SQ 29,548.

Influence of ketanserin and ritanserin on CFR frequency. Results are presented in table 3 and figures 2 and 3. Administration of either ketanserin or ritanserin, 15 min after initiation of CFRs, dose-dependently reduced CFR frequency over the first 30 min of observation, with significant inhibition from 40 and 20 μg/kg, respectively (both $P < .05$), giving ID_{50} values of 89 μg/kg (95% confidence limits, 36–286 μg/kg) and 77 μg/kg (95% confidence limits, 40–132 μg/kg), respectively. The highest doses of ketanserin and ritanserin (2500 μg/kg) abolished CFRs in four of five and six of six rabbits, respectively, and produced maximal reductions in CFR frequency of 83 ± 7% and 98 ± 2% (both $P < .05$ *vs.* base line). MAP was significantly reduced by the high dose of ketanserin (ΔMAP = −21 ± 7 mm Hg; $P < .05$ *vs.* vehicle group), whereas no significant reduction was observed in ritanserin-treated, compared with vehicle-treated, animals. HR was statistically significantly reduced by the high dose (2500 μg/kg) of ketanserin and ritanserin (ΔHR = −36 ± 22 and −36 ± 9 beats/min respectively; $P < .05$ *vs.* vehicle group). In additional experiments, local infusion of exogenous 5-HT (20.8 μg/kg/min, $n = 11$) in rabbits pretreated with 160 μg/kg ritanserin restored CFR frequency to vehicle group levels (from 22.2 ± 1.4 to 10.9 ± 2.0 and then 17.1 ± 3.7 cycles/hr, at base line, first and second period, respectively, of the 5-HT infused group; P = NS for second period *vs.* base line and vehicle group; $P < .05$ for second period *vs.* first period; Fig. 3B). 5-HT was also unable to restore CFRs in three rabbits whose CFRs had been abolished by ritanserin.

Influence of 5-$HT_{1B/D}$ and *alpha*-1 adrenergic receptor blockade and COX inhibition on CFR frequency. Because both ketanserin and ritanserin have affinity for 5-$HT_{1B/D}$ receptors, we addressed the possibility that inhibitory activities of both compounds on CFR frequency could be mediated through 5-$HT_{1B/D}$ receptor blockade. For this purpose, we determined whether CFR frequency could be reduced by the novel and highly selective 5-$HT_{1B/D}$ receptor antagonist GR 127935. Administration of GR 127935 (630 μg/kg i.v.) did not statistically significantly reduce CFR frequency or modify MAP or HR, compared with vehicle-infused animals (table 3).

To further evaluate whether the activity of ketanserin on CFR frequency could be related to its *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive effects, we explored whether CFR frequency could be reduced by the *alpha*-1 adrenoceptor antagonist prazosin, at a dose (160 μg/kg i.v.) producing systemic hypotension equivalent to that induced by the highest dose of ketanserin studied (ΔMAP = −29 ± 5 *vs.* −21 ± 7 mm Hg; both $P < .05$ *vs.* vehicle-treated rabbits and P = NS between groups). Under these conditions, prazosin did not statistically significantly reduce CFR frequency, with respect to vehicle-treated animals (table 3).

Finally, to verify the platelet dependency of CFRs under our experimental conditions, we determined whether CFR frequency could be reduced by the COX inhibitor aspirin. Acute i.v. administration of aspirin, 15 min after initiation of CFRs, dose-dependently reduced CFR frequency over the first 30 min of observation, by 59 ± 21 and 92 ± 3% at 2,500 and 10,000 μg/kg, respectively, without statistically significantly affecting MAP or HR (table 3).

Discussion

The present studies performed in anesthetized rabbits demonstrated that the TP receptor antagonist SQ 29,548 dose-dependently inhibited thrombus formation in both low- and high-shear rate arterial thrombosis, whereas ketanserin and ritanserin were effective only in the high-shear rate model of CFRs. The damping activity of ketanserin and ritanserin on CFR frequency could not be attributed to 5-$HT_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive activities but, rather, was attributed to 5-$HT_{2A/C}$ receptor antagonist properties. Furthermore, local infusions of either the TxA_2 analog U-46619 or 5-HT to animals pretreated with SQ 29,548 or ritanserin, respectively, restored CFR frequency to vehicle-infused levels. These results strongly suggest that TP receptors are involved in arterial thrombosis in rabbits independently of the shear rate, whereas 5-$HT_{2A/C}$ receptors play a major role only in high-shear rate thrombus formation.

TABLE 3

Influence of drugs on CFR frequency and hemodynamic parameters

Values are mean ± S.E.M. SQ 29,548, GR 127935 and prazosin were used to block TP, 5-$HT_{1B/D}$ and *alpha*-1 adrenoceptors, respectively. ID_{50} refers to the geometric mean antagonist dose [with 95% confidence intervals (CI) in parentheses] inhibiting responses by 50%. Absolute changes in MAP and HR were determined between time 30 min and base line.

Number	Body Weight	Treatment	Dose	CFR	ID_{50} (95% CI)	MAP		HR	
						Base line	Absolute Changes	Base line	Absolute Changes
	kg		μg/kg	% inhibition	μg/kg	mm Hg		beats/min	
15	2.6 ± 0.1	Vehicle	1 ml/kg	10 ± 3		89 ± 3	−8 ± 3	285 ± 7	−3 ± 5
6	3.0 ± 0.1	SQ 29,548	10	10 ± 5	35 (24–58)	76 ± 4	−10 ± 4	270 ± 17	2 ± 11
6	2.7 ± 0.1	SQ 29,548	20	28 ± 14		83 ± 7	−15 ± 4	269 ± 20	−20 ± 10
10	2.6 ± 0.0	SQ 29,548	40	61 ± 12*		73 ± 5	−4 ± 4	249 ± 8	−3 ± 7
8	2.6 ± 0.1	SQ 29,548	160	80 ± 8*		66 ± 6	−1 ± 4	272 ± 9	−3 ± 5
4	2.8 ± 0.1	Ritanserin	10	16 ± 4	77 (40–132)	74 ± 10	−5 ± 3	253 ± 27	−25 ± 10
6	2.7 ± 0.0	Ritanserin	20	37 ± 15*		83 ± 5	−15 ± 3	280 ± 18	−7 ± 9
6	2.8 ± 0.0	Ritanserin	40	38 ± 7*		77 ± 5	−3 ± 2	260 ± 8	−7 ± 4
13	2.6 ± 0.1	Ritanserin	160	59 ± 9*		66 ± 8	−12 ± 3	270 ± 10	−4 ± 5
5	2.4 ± 0.2	Ritanserin	630	83 ± 8*		69 ± 10	−2 ± 10	251 ± 30	−18 ± 9
6	2.4 ± 0.1	Ritanserin	2,500	98 ± 2*		80 ± 5	−9 ± 4	271 ± 14	−36 ± 9*
4	2.8 ± 0.1	Ketanserin	10	14 ± 5	89 (36–285)	88 ± 11	−3 ± 6	239 ± 14	−15 ± 14
5	2.5 ± 0.1	Ketanserin	40	48 ± 15*		74 ± 2	−1 ± 5	254 ± 17	−15 ± 5
5	2.8 ± 0.0	Ketanserin	160	56 ± 14*		93 ± 10	−9 ± 3	273 ± 13	−18 ± 7
6	2.4 ± 0.1	Ketanserin	630	74 ± 16*		100 ± 9	−8 ± 5	277 ± 13	−1 ± 10
5	2.4 ± 0.1	Ketanserin	2,500	83 ± 7*		86 ± 5	−21 ± 7*	279 ± 16	−36 ± 22*
9	2.7 ± 0.0	GR 127935	630	21 ± 6		65 ± 5	3 ± 2	233 ± 11	−6 ± 5
5	2.3 ± 0.1	Prazosin	160	29 ± 14		82 ± 4	−29 ± 5*	272 ± 8	−8 ± 7
3	2.9 ± 0.1	Aspirin	2,500	59 ± 21*	<2500	102 ± 1	−12 ± 4	291 ± 11	9 ± 9
5	2.1 ± 0.0	Aspirin	10,000	92 ± 3*		88 ± 4	−14 ± 4	251 ± 10	−2 ± 9

* $P < .05$ *vs.* vehicle-treated group.

Involvement of TP and 5-$HT_{2A/C}$ receptors in high-shear rate arterial thrombosis. In the carotid stenosis and endothelial injury model, SQ 29,548, ritanserin and ketanserin all dose-dependently reduced CFR frequency (ID_{50} values of 35, 77 and 89 μg/kg, respectively). The inhibitory activity of ketanserin and ritanserin on CFRs could not be attributed to either 5-$HT_{1B/D}$ or *alpha*-1 adrenoceptor antagonist properties and associated systemic hypotensive activities (see below) but, rather, was attributed to 5-$HT_{2A/C}$ receptor antagonist properties. Local infusion of U-46619, a stable TxA_2 analog, or 5-HT at the site of the injury restored CFR frequency to vehicle-infused levels in animals whose CFRs had been reduced, but not abolished, by SQ 29,548 or ritanserin, respectively. These results provided further evidence that TP and 5-$HT_{2A/C}$ receptors mediated thrombus formation and maintenance in the CFR experiments, in agreement with previous reports (Willerson *et al.*, 1989; Golino *et al.*, 1990, 1992, 1993; Torr *et al.*, 1990; Salvati *et al.*, 1993; Beaughard *et al.*, 1995). Furthermore, aspirin also dose-dependently reduced CFR frequency, thus confirming the platelet-dependent CFR occurrence described in dogs with coronary artery stenosis and endothelial injury (Folts, 1995).

In addition to possessing nanomolar affinity for 5-$HT_{2A/C}$ receptors (for reviews, see Zifa and Fillion, 1992; Hoyer *et al.*, 1994), ketanserin and ritanserin also have affinity for 5-$HT_{1B/D}$ receptors (Weinshank *et al.*, 1991; Pauwels *et al.*, 1995). Therefore, we addressed the possibility that inhibitory activities of ketanserin and ritanserin on CFR frequency could be mediated through 5-$HT_{1B/D}$ receptor antagonism. The novel and highly selective receptor antagonist GR 127935 (Clitherow *et al.*, 1994; Skingle *et al.*, 1994), at a dose (630 μg/kg i.v.) that is higher than that required to fully block 5-$HT_{1B/D}$ receptor-mediated carotid vasoconstriction in anesthetized pigs (De Vries *et al.*, 1996), did not alter CFR frequency or hemodynamic parameters. Thus, these results provide evidence that 5-$HT_{1B/D}$ receptors are apparently not involved in mediating thrombus formation under the present experimental conditions.

The damping activity of ketanserin on CFR frequency cannot be related to *alpha*-1 adrenoceptor antagonist properties (Leysen *et al.*, 1981) either, because the selective *alpha*-1 adrenoceptor antagonist prazosin, at a dose producing systemic hypotension equivalent to that induced by the highest dose of ketanserin (2,500 μg/kg), did not reduce CFR frequency. These results suggest that *alpha*-1 adrenoceptors are not involved in mediating arterial thrombus formation under high-shear rate conditions.

Both TxA_2 and 5-HT are implicated in the pathogenesis of platelet aggregation and dynamic vasoconstriction that occur at sites of endothelial injury and coronary artery stenosis. When platelets aggregate, they release (among other factors) 5-HT, which causes local vasoconstriction and acts to amplify and further promote aggregation. It might be expected that vasodilation (reflected by systemic hypotension), as observed after administration of the highest dose of ketanserin investigated, would counteract the local vasoconstriction induced by the vasoactive agents released during aggregation and would thus reduce further aggregation (*i.e.*, reduce CFR frequency). This possibility can be excluded because 1) a statistically significant reduction in CFR frequency was observed at doses of ketanserin that did not affect MAP (≤630 μg/kg), 2) ritanserin at a dose that reduced CFR frequency to the same extent as that produced by the highest dose of ketanserin (83 ± 8% at 630 μg/kg *vs.* 83 ± 7% at 2,500 μg/kg) did not reduce MAP and, 3) at a dose producing systemic hypotension equivalent to that evoked by the highest dose of ketanserin, the *alpha*-1 adrenoceptor antagonist prazosin did not significantly reduce CFR frequency. Moreover both ketanserin and ritanserin, at the highest dose investigated (2,500 μg/kg), induced bradycardia, which could reduce cardiac output, as reported by Bolt and Saxena (1985), and possibly carotid blood flow. In fact, ritanserin at a dose that reduced CFR frequency to the same extent as that produced by the highest dose of ketanserin (83 ± 8% at 630 μg/kg *vs.* 83 ± 7% at 2,500 μg/kg) did not alter HR, thus excluding bradycardia as a major antithrombotic mechanism of action of these drugs. Taken together, these results provide evidence that the 5-$HT_{1B/D}$ and *alpha*-1 adrenergic antagonist properties of ketanserin and ritanserin are not involved in reducing CFR frequency, thus confirming that both 5-$HT_{2A/C}$ and TP receptor activation are major mechanisms involved in CFR occurrence and maintenance in stenotic and endothelially injured rabbit carotid arteries.

Differential involvement of TP and 5-$HT_{2A/C}$ receptors in low-shear rate arterial thrombosis. In contrast to ketanserin and ritanserin, SQ 29,548 significantly and dose-dependently inhibited arteriovenous shunt occlusion without affecting hemodynamic parameters. Inactivity of ketanserin and ritanserin in the present experimental model cannot be explained by the use of inadequately low doses, because 2,500 μg/kg is relatively high, compared with doses that substantially inhibit 5-$HT_{2A/C}$ receptor-mediated responses *in vivo* (Bolt and Saxena, 1985; Pettersson *et al.*, 1985; Docherty, 1989; Valentin *et al.*, 1995). Antithrombotic inactivity of ketanserin has previously been reported in an arteriovenous shunt model in rats (Maffrand *et al.*, 1988). In addition, such doses of ketanserin and ritanserin are likely to have extensively blocked *alpha*-1 adrenergic (Bolt and Saxena, 1985; Pettersson *et al.*, 1985; Docherty, 1989) or 5-$HT_{1B/D}$ (De Vries *et al.*, 1996) receptors, respectively (see above), thereby excluding any involvement of *alpha*-1 adrenoceptors or 5-$HT_{1B/D}$ receptors in arterial thrombus formation under low-shear rate conditions. 5-$HT_{2A/C}$ and TP receptors clearly do not share similar involvement in mediating arterial thrombus formation under low-shear rate conditions. Moreover, the antithrombotic effectiveness of SQ 29,548 can be accounted for by inhibition of locally produced TxA_2/endoperoxides, which elicit platelet aggregation (Ogletree, 1987). The extracorporeal arteriovenous shunt was previously described in rats as a platelet-predominant thrombosis model (Umetsu and Sanai, 1978; Shand *et al.*, 1984), and the histological analyses of thrombus composition we performed (data not shown) confirm and extend these observations to rabbits. Evidence is therefore presented that platelet activation is a key component of arterial thrombus formation under low-shear rate conditions in the rabbit arteriovenous shunt. Interestingly, no information is currently available on the existence of putative platelet 5-$HT_{1B/D}$ receptors. Thus, platelet activation is mediated partly through TP receptor stimulation, whereas platelet 5-$HT_{2A/C}$ receptors appear to have little or no involvement.

Thrombus formation in high- *vs.* low-shear rate arterial thrombosis. A major finding of the present study was that SQ 29,548 elicited antithrombotic activity independently of the shear rate, whereas ketanserin and ritanserin exerted substantial antithrombotic activity only when shear rates were high. High shear rates, such as those found in the present study, are not physiological (20,000–60,000 sec^{-1}) but are reached under pathological conditions in stenotic arteries (Goldsmith and Turitto, 1986; Strony *et al.*, 1993). The way in which shear stress can induce aggregation of platelets is gradually being elucidated. It is now established that the GP IIb/IIIa receptor, the final common pathway of platelet aggregation, interacts only with fibrinogen in a low-shear rate environment, whereas it interacts mainly with vWf in a high-shear rate environment (Ruggeri, 1994). The role of vWf appears to be most significant at high shear rates, presumably as a consequence of its unique molecular architecture. Under the effects of high shear forces, vWf molecules take the shape of extended filaments; the repeating subunit structure of these large multimers offers an array of interaction sites capable of binding in a multivalent manner to receptors on the platelet membrane, thereby increasing the number of contact points and the strength of interaction. As a result, the overall force linking platelets to the surface and/or to one another is increased. This interpretation of events explains why the role of vWf is less relevant at lower shear rates, because other adhesive molecules may provide sufficient force of interaction to withstand opposing shear forces of lesser magnitude (Chow *et al.*, 1992; Ikeda *et al.*, 1993; Ruggeri, 1994). In remarkable contrast, GP IIb/IIIa in a low-shear rate environment shows the ability to interact only with immobilized fibrinogen (Savage and Ruggeri, 1991). Interestingly, monoclonal antibodies directed against GP IIb/IIIa and GP IIb/IIIa receptor antagonists have demonstrated high efficacy in situations of both low (Ikeda *et al.*, 1991) and high (Coller and Scudder, 1985; Gold *et al.*, 1988; Shebuski *et al.*, 1989a,b; Chow *et al.*, 1992) shear rates, confirming the pivotal role of GP IIb/IIIa in the process of thrombus formation, independently of shear rate. Furthermore, Golino *et al.* (1995) recently demonstrated the key role of GP IIb/IIIa in the stenotic and endothelially injured rabbit carotid artery model (Golino *et al.*, 1995).

Differential antithrombotic effectiveness of ketanserin and ritanserin, even at relatively high doses (Valentin *et al.*, 1995), is also in agreement with different mechanisms mediating thrombus formation at high *vs.* low shear rates and strongly suggests that 5-HT plays a major role in thrombus growth only under high-shear rate conditions. A role for 5-HT in mediating the formation of arterial thrombi under low-shear rate conditions cannot, however, be excluded, because the indoleamine has been reported to mediate platelet aggregation *in vitro*, albeit weakly, and to contribute to aggregate growth *in vivo* (Menys, 1993). The basis for a major role of 5-HT in high-shear rate thrombus formation, compared with a minor role with low shear rates, is unclear at present but could involve enhanced 5-HT release from platelet dense granules with high shear rates. It is well established that, *in vitro*, high shear stresses (>50 dyn/cm^2) activate spontaneous or agonist-induced aggregation by the release of platelet dense granule contents (Brown *et al.*, 1975). This would be compatible with the increased transcardiac 5-HT concentrations that have been observed in patients with coronary stenoses (Van den Berg *et al.*, 1989). Factors other than shear rate may have influenced the differential responsiveness of 5-$HT_{2A/C}$ receptor antagonists in the two models. Differences in 1) thrombogenic substrate between the two models (*i.e.*, silk thread *vs.* exposed subendothelial collagen) and 2) the size of the thrombogenic surface may also be involved in the differential antithrombotic effectiveness of ketanserin and ritanserin, which would also lend support to the hypothesis

that the mechanisms of thrombus formation, in particular platelet activation, are different under high- *vs.* low-shear rate conditions. In contrast to the 5-$HT_{2A/C}$ receptor, TP receptor activation plays a major role in both high- and low-shear rate arterial thrombosis. A possible explanation is that arachidonic acid metabolism in platelet membranes, leading to TxA_2/endoperoxide production and consequent TP receptor activation, occurs independently of the mechanism of platelet activation *in vivo* (Badimon *et al.*, 1992; Reilly and FitzGerald, 1993). Although 5-$HT_{2A/C}$ and TP receptor antagonists have demonstrated efficacy in preventing thrombus formation in high-shear rate situations, the relative physiological and pathological importance of these mechanisms is probably moderate, compared with functional antagonism of GP IIb/IIIa.

In conclusion, our results indicate that TP receptors are involved in arterial thrombosis in rabbits, independently of the shear rate, whereas 5-$HT_{2A/C}$ receptors play a role only in high-shear rate thrombus formation. The precise mechanism underlying the differential role of 5-$HT_{2A/C}$ receptors in high- *vs.* low-shear rate arterial thrombosis deserves further study.

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(Fig. 1g,h). A few True blue-positive neurons in GEG also contained substance P or calcitonin gene-related peptide. None of the ganglia examined accumulated True blue after injection of the dye in the mimic muscles.

Some cells in SPG accumulated True blue after palate injection, a few of which were GRP-positive. No cells of the internal carotid ganglion contained True blue following application to the tongue or palate. Trigeminal ganglion cells accumulated True blue, many of which were positive for substance P and calcitonin gene-related peptide, after dye application to the tongue, palate and skin. Following True blue application to the middle cerebral artery no dye accumulation was found in GEG, but in choline acetyltransferase-, vasoactive intestinal polypeptide-, neuropeptide Y-, substance P- and calcitonin gene-related peptide-positive cells in the SPG, otic, trigeminal and internal carotid ganglia.

The GRP antiserum cross-reacts with bombesin. Therefore, the identity of GRP in GEG from rat (pooled from both sides of 3 animals) was confirmed by HPLC (Fig. 2). Material displaying GRP in the tissue extracts was analyzed by reversed-phase HPLC on a Waters model 204 liquid chromatograph equipped with a model U6K injector and an absorption detector 441, a model 6000 A pump, an M-45 pump and an automated gradient controller. An Aquapore RP-300 column (Brownlee labs, St. Clara, USA) was used. The samples were eluted with CH_3CN and 0.08% trifluoroacetic acid (v/v) pH 2.5, using a linear gradient of CH_3CN (20–32.5%) during the first 25 min followed by 10 min of isocratic elution. Fractions of 0.5 ml were collected, lyophilized and assayed for GRP. The content of GRP-like peptide(s) in GEG, SPG, trigeminal ganglion and pial vessels, pooled from 3 rats, was measured by radioimmunoassay. The content was considerably higher in GEG (43.0 pmol/g) than in SPG (12.4 pmol/g), trigeminal ganglion (1.2 pmol/g) and pial vessels (6.9 pmol/g).

The study demonstrates the presence of authentic GRP in the great majority of neurons in GEG of rat. Also in the human GEG GRP-positive neurons were found. As expected, True blue, applied to areas rich in taste buds, accumulated in neurons of this ganglion. These cells were all GRP-positive. However, the same peptide was not found in nerve fibers in the tongue or palate (see also ref. 7) or along the likely pathways for gustatory fibers, i.e. the chorda tympani from the tongue and the Vidian nerve and GSPN (after passage through the SPG) from the palate. At least some of the auricular cutaneous fibers also originate in GRP-containing neurons of the GEG, as presently demonstrated (see also ref. 10). The inability to demonstrate GRP in nerve fibers may have technical explanations, i.e. the antiserum may not be able to visualize low concentration of the peptide. Alternatively, GRP may not be transmitter in a specific subgroup of GEG neurons (gustatory neurons) but may subserve other functions in the neurons, like being a trophic factor (see also ref. 15).

No evidence could presently be found in rat for a deep sensory innervation to mimic musculature of the facial nerve, as hypothetized by Keller and van Loveren to be present in man [6]. No immunohistochemical evidence for an innervation by GRP fibers of cerebral vessels could be obtained, in contradiction to one report on pial arteries from mouse, rat, guinea-pig and cat [13]. In line with this, only low GRP-like activity was measured by radioimmunoassay in pial vessels and the trigminal ganglion as compared to GEG.

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Serotonergic excitatory drive to hypoglossal motoneurons in the decerebrate cat

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In decerebrate, paralyzed, vagotomized and artificially ventilated cats, serotonin (5-HT) and its analogues, microinjected into the hypoglossal (XII) motor nucleus, altered the activity of the genioglossal branch of XII nerve. 5-HT, carboxamidotryptamine maleate (5-CT) and DOI (1–5 mM) increased the activity by over 200%. Methysergide reversed this increase. Methysergide, mianserin, or ketanserin (100–250 nl, 1 mM) reduced the spontaneous hypoglossal activity by 20–50%. Buspirone, 8-OH-DPAT and (−)-propranolol were without effect. Thus, 5-HT provides a substantial tonic excitatory drive to XII motoneurons. The 5-HT receptors involved are likely to be type 1C or 2, but uncertainty regarding the affinity profiles of the drugs used in in vivo conditions in the cat precludes a definite identification.

Serotonergic neurons of the brainstem raphe nuclei form extensive networks throughout the central nervous system and function in the control of a diversity of physiological and behavioral activities (autonomic and somatic, sensory and motor). There is now considerable evidence that serotonin (5-HT) has a predominantly excitatory action on motoneurons located at various levels of the neuraxis (e.g., 6, 9, 21, 24, 25; see ref. 10 for more refs.). Different 5-HT receptor subtypes and ionic conductances are involved (see refs. 3, 26 for reviews). This excitatory effect is often related to the role played by 5-HT in the maintenance of motor activity during the waking state (cf., refs. 7, 10). A possible serotonergic excitatory drive to respiratory motoneurons is of special interest as it is likely to decrease during REM sleep [17, 27] thereby contributing to the sleep-related depression of breathing, including the atonia of upper airways (e.g., the sleep apnea syndrome). In support of this reasoning, there is evidence that phrenic and laryngeal motoneurons are excited by 5-HT [9, 18, 25] (see ref. 30 for more refs.). In addition, both facial and trigeminal motoneurons are facilitated by iontophoretically applied 5-HT [13, 15, 16, 24]. Consequently, we hypothesize that a withdrawal of the serotonergic excitatory drive during sleep could result in disfacilitation of cranial motoneurons involved in the maintenance of upper airway patency, thereby leading to sleep-related obstructions. We chose to study hypoglossal (XII) motoneurons because they are well characterized, representative upper airway motoneurons, that are important in the control of airway patency (see ref. 4 for a review). Both 5-HT terminals [1] and receptors [22] are present within the XII motor nucleus. Thus, the nucleus itself is an important site where 5-HT may exert its effects on XII motoneurons. The character and strength of these local effects have not been studied in in vivo conditions. Therefore, to begin addressing our hypothesis, we assessed: (1) the effects mediated by 5-HT receptors located within the XII motor nucleus in unanesthetized, decerebrate cats; (2) which 5-HT receptors may underlie the effects; and (3) whether the spontaneous activity of XII motoneurons is subjected to a tonic serotonergic excitatory drive.

Experiments were performed on 23 adult cats of either sex, weighing 1.8–3.1 kg. The animals were preanesthetized with ketamine (80 mg, i.m.) and diazepam (2 mg, i.m.), anesthetized with halothane, and decerebrated at a precollicular level. The dissection and recording procedures were the same as in a previous report from this laboratory [14]. The genioglossal branches of the XII

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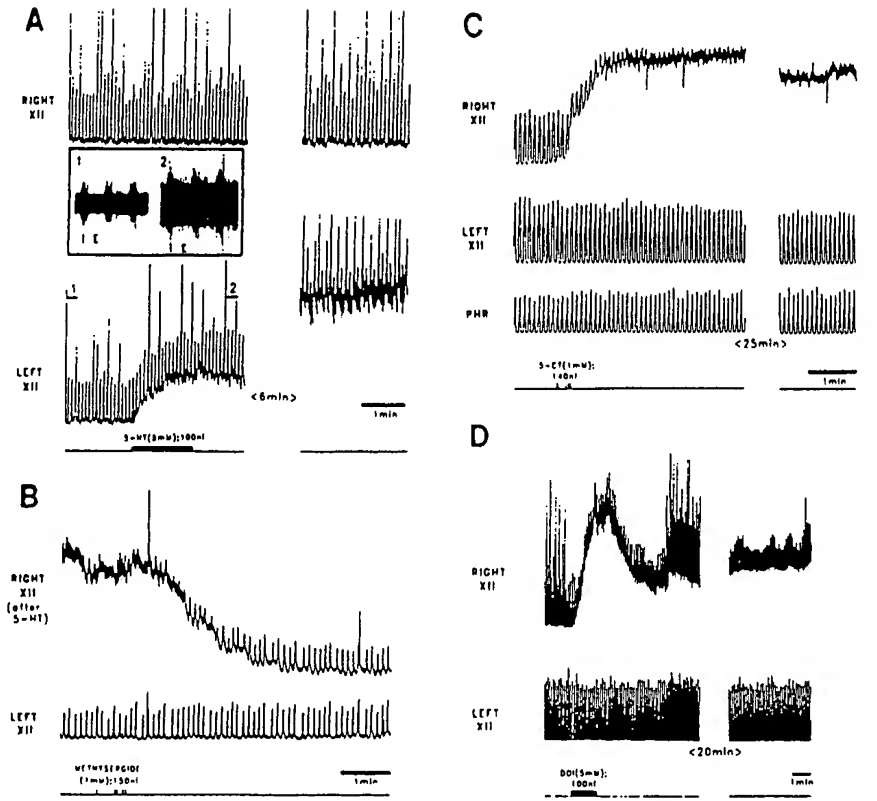

Fig. 1. Effects of microinjections of serotonergic agonists. The records show the moving averages of hypoglossal (XII) nerve activities. The inset in A shows, on an expanded time scale, the raw records of the XII nerve activity before and after treatment with 5-HT. Inspiratory and expiratory phases are marked by I and E, respectively. Note also the phrenic nerve moving average record (PHR) in C which marks each inspiration. The control XII nerve activities consisted of a relatively weak tonic component with strong inspiratory bursts, as shown in relation to phrenic nerve activity in part C. A: injection of 5-HT into the left XII nucleus, with the right XII as control. Injection marked by a thick bar at bottom. The excitatory effect reached a steady state after about 6 min (right side). Note that the respiratory modulation of the XII nerve on the injected side was reduced during the prominent increase in tonic activity. Note that the 'swallow-like' bursts of activity (occurring at the end of many inspiratory bursts) were present bilaterally both before and after 5-HT microinjection, thus indicating that the microinjection did not disrupt this spontaneous behavior of XII motoneurons. B: methysergide reversal of the excitatory effect of 5-HT (150 nl, 5 mM) produced 80 min earlier in the motoneurons of the right XII nerve (injection marked by the pressure pulse marker in the bottom trace). Note the restoration of the pattern of firing of the nerve on the treated side. This is a different experiment from that shown in A. C: microinjection of 5-CT into the right XII nucleus (pulse marker in bottom trace) resulted in a strong enhancement of XII nerve activity. It was maintained with only a minor adaptation for over 30 min (right side). D: microinjection of DOI into the right XII nucleus also had an excitatory effect. The records show activities during the injection (left) and 20 min later (right).

nerve were prepared bilaterally for recording, in addition to one phrenic nerve (C_5 root) and a motor branch innervating neck muscles (C_4). The animals were vagotomized, paralyzed (gallamine triethiodide, continuous infusion of 5 mg/kg/h) and artificially ventilated with an inspired O_2 of 40%. To maintain a regular respiratory modulation of XII nerve activity, CO_2 was added to the inspired gas mixture. The end-expiratory CO_2 in different animals was 3.5–4.5%. In individual animals, it was kept constant throughout the experiment. Blood pressure and temperature were continuously monitored and remained within physiological limits.

The medulla was exposed and the anterior cerebellar vermis reflected rostrad to uncover the region of the XII motor nucleus. Drug filled, single-barrel pipettes (tip diameter: 15–20 μm) were connected to a pressure pulse source and an amplifier and inserted into the lower brainstem, aiming at the center of the XII motor nucleus just rostral to the obex where the majority of genioglossal motoneuronal cell bodies are located [28]. Recording of inspiratory-modulated neural activity at the pipette tip aided in optimal placement of the pipette for injection. To assure that the recorded activity originated in XII motoneurons with axons in the dissected XII nerve branch, the activity of the branch was averaged using as triggers action potentials recorded with the pipette in the motor nucleus. The microinjections were performed at sites from which the averaged record showed sharp action potentials having a latency compatible with orthodromic conduction in axons of XII motoneurons. The injected volumes were directly determined by measuring the movement of the meniscus in the pipette with a pocket microscope and reticle. A single injection of a given drug, in a volume of 100–250 nl in most cases, was performed in each nucleus. The peak amplitude of the moving average of nerve activities was measured with respect to the baseline (determined during periods when no action potentials were seen on the electroneurograms) and used to characterize the effects of microinjections. The effects of the microinjections were considered to be localized to the XII nucleus if the simultaneously recorded activity of the contralateral XII nerve and the phrenic nerve remained within ±10% for at least 20 min following the injection. In preliminary experiments with agonists having a strong effect (e.g., 5-CT, see below), we have determined that injections larger than 250 nl resulted in some evidence of the spread of the drug to the contralateral XII nucleus within 30–60 min. Based on this observation, the data reported here are only from those experiments in which there were no signs that the drugs spread beyond the boundaries of the XII nucleus.

The following drugs were used: 5-HT creatinine sulfate (Sigma), carboxamidotryptamine maleate (5- CT), (±)-DOI HCl, 8-OH-DPAT-HBr, buspirone, mianserin HCl, ketanserin tartrate, *S*-(−)-propranolol HCl (all from R.B.I.) and methysergide maleate (Sandoz). They were prepared in saline 2–3 h before use in concentrations of 0.1–5 mM for agonists and 1 mM for antagonists.

Microinjections of 5-HT (n=3 experiments; 50–125 nl; 5 mM) caused a large increase of the peak XII nerve activity to 220%±63 (S.D.) of control (Fig. 1A). This increase was due to an increase in the tonic activity of the motoneurons, while the respiratory modulation of activity usually decreased as the tonic excitation increased. This excitation was reversed by subsequent local methysergide, a non-selective 5-HT antagonist, injections (n=3; 150–240 nl) and the respiratory modulation reappeared (Fig. 1B). 5-CT, a non-selective 5-HT_1 agonist, (n=5; 100–140 nl; 1 mM) enhanced the activity to 234%±19 (S.D.) of control and also strongly reduced the respiratory modulation (Fig. 1C). Subsequent microinjections of methysergide (n=2; 120–200 nl) reversed the 5-CT effect. In one experiment, 0.1 mM 5-CT (100 nl) increased the activity to 190% of control. Neither buspirone (n=2; 100 nl; 2 mM) nor 8-OH-DPAT (n=2; 100–120 nl; 1–2 mM), selective 5-HT_{1A} agonists, had an excitatory effect.

DOI, primarily a 5-HT_2 agonist, also had an excitatory effect on XII motoneurons. In three experiments with three different concentrations of the drug used, the activity was enhanced to 123% (420 nl; 1 mM), 154% (150 nl; 2 mM), and 220% (100 nl; 5 mM) of control (Fig. 1D). Thus, compared to the potent excitatory effects of 5-CT obtained at concentrations of 0.1–1.0 mM, the effects of DOI appeared to be weaker. In two experiments, ketanserin (200 nl), primarily a 5-HT_2 antagonist, reduced the excitatory effect of DOI, although a complete reversal was not obtained even after 30 min.

Methysergide injected in control conditions (n=7; 160 nl±80 (S.D.) (range 40–250 nl)) often (5/7) resulted in an initial, transient excitation followed in all seven experiments by a depression that averaged 57%±14 (S.D.) of control (range: 36–72%) (Fig. 2). Mianserin, a 5-$HT_{1C,2}$ antagonist, (n=3; 100–200 nl) depressed the spontaneous activity to 72%±12 (S.D.) of control. Ketanserin (n=2) reduced the spontaneous XII nerve activity to 67% (80 nl) and 84% (150 nl) of control. A transient excitation before the depression developed was sometimes observed with these last two drugs. Propranolol, a 5-HT_{1A} and β-adrenergic antagonist, (n=2; 100 nl) had no effect on the spontaneous activity nor could it reverse the excitatory effect of 5-CT (one experiment).

This study demonstrates that there is an excitatory effect mediated by 5-HT receptors located within the XII nucleus on XII motoneurons in a decerebrate, unanesthetized cat. By the use of antagonist injections alone, we found that there is an endogenous serotonergic excitatory drive to XII motoneurons, a finding similar to that for phrenic motoneurons [25]. The identification of the 5-HT receptor type involved and its specific cellular location will require further studies that can now be guided by the present experiments.

With regard to the location of the receptor, several precautions discussed above, were employed to deliver the drugs as close as possible to the cell bodies of genioglossal motoneurons and to minimize spread beyond the nucleus. As a result, we observed rapid responses following microinjections of much smaller amounts of the agonists than those used in similar studies of the effects of 5-HT on phrenic [25] and trigeminal [24] motoneurons. Thus, the receptors mediating these

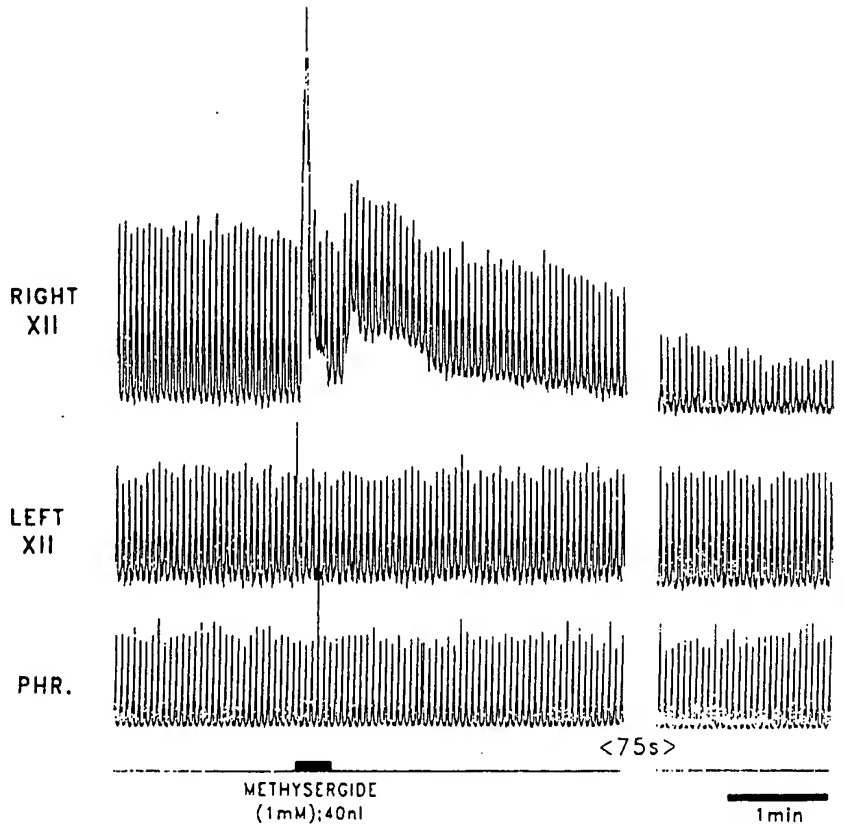

Fig. 2. Effect of methysergide microinjection placed in the right XII nucleus on the spontaneous activity of the XII nerve. Injection marked by the thick bar in the bottom trace. Note the initial excitation followed by a marked depression of activity on the treated side. The end of the record on the right side shows the maximal depression attained. It was then maintained for several hours. Traces as in Fig. 1.

effects must be located within the boundaries of the XII nucleus. They may be located postsynaptically on XII motoneurons as 5-HT containing terminals were found in close apposition with dendrites of inspiratory-modulated XII motoneurons [12].

Our study using different agonists and antagonists implicates 5-$HT_{1C,2}$ receptors in mediating the excitatory effects to XII motoneurons. This conclusion is based on the fact that two relatively selective 5-$HT_{1C,2}$ antagonists, mianserin and ketanserin, produced a reduction of the spontaneous XII nerve activity, whereas methysergide, a non-selective antagonist, had only a slightly higher potency. In addition, DOI, primarily a 5-HT_2 agonist, had a substantial excitatory effect, although not stronger than 5-HT itself. Of all the agonists used, the strongest excitation was evoked by 5-CT, a nonselective 5-HT_1 agonist, with even a 100 μM solution having a powerful effect. This would appear to indicate the involvement of 5-HT_{1A} receptors. However, two selective 5-HT_{1A} agonists, buspirone and 8-OH-DPAT, were ineffective, as was propranolol, a 5-HT_{1A} and β-adrenergic antagonist. Taken together, these data suggest that the receptors mediating the excitatory effect on XII motoneurons are of type 1C/2 or a functionally similar but pharmacologically distinct receptor type proposed recently to mediate excitation of spinal motoneurons [5; cf. 3]. It is of interest that both 5-CT and a relatively selective 5-HT_2 agonist (DOM) had excitatory effects on facial motoneurons that could be antagonized by 5-$HT_{1C,2}$ antagonists [23]. Thus, these results are similar to ours with XII motoneurons. The particularly strong effect of 5-CT observed in our studies may be related to a relatively high density of 5-HT_{1C} receptors within the XII nucleus [22] and/or 5-CT's ability to act as 5-HT releaser [29]. A quantitative addressing of the issue of relative potencies of drugs is difficult in in vivo microinjection experiments because the resolution of agonist-antagonist interaction studies is technically limited by the long duration of the effect (hours, cf. refs. 23–25) and by the fact that a uniform distribution of the drugs within the nucleus can never be obtained. Moreover, the assumptions regarding the relative affinities of the drugs used are based on in vitro studies (mostly using the competitive binding technique in the rat brain) while the performance of these drugs in vivo in the cat may be different and/or modified by receptor interactions (cf. refs. 3, 8, 26). Thus, further investigation awaits the development of selective 5-HT_{1C} drugs with known properties in in vivo conditions.

Our conclusion that 5-HT is excitatory to XII motoneurons is opposite to that reached by another group in their recent in vitro studies on XII motoneurons in the isolated brainstem–spinal cord preparation from neonatal rats [19,20]. These studies provided evidence for an inhibitory effect of 5-HT on XII motoneurons. In these studies, the drugs were applied in the superfusing medium and therefore all the neurons in the brainstem could be affected. In the light of our results, this discrepancy suggests that 5-HT may exert an inhibitory effect on XII motoneurons indirectly, by acting on as yet undetermined inputs to XII motoneurons located at sites remote from the XII nucleus. In support of this interpretation, recent studies using neonatal rat brainstem slices also showed an excitatory rather than inhibitory effect of 5-HT on XII motoneurons [2].

The specificity of the depressant effects of 5-HT antagonists on spontaneous XII nerve activity requires further study. It is noteworthy, however, that in many iontophoretic studies methysergide or ritanserin in amounts sufficient to block the 5-HT excitatory effects did not attenuate the excitatory effects of norepinephrine [10, 13, 23]. Likewise, in our related studies, a long-lasting facilitation of XII nerve activity produced by electrical stimulation of the superior laryngeal nerve [cf. ref. 18] was maintained following microinjections of methysergide similar to those used in the present study (Tojima, Kubin and Davies, unpublished observations). Therefore, we interpret the depressant effect of 5-HT antagonists found in this study as being specifically related to their action on 5-HT receptors located within the XII nucleus.

The excitatory effect of 5-HT on XII motoneurons found in this study permits us to add this group of motoneurons to a growing list of those subjected to tonic 5-HT facilitatory effects. Interestingly, in a few recent studies using unanesthetized animals, including the present study, 5-HT alone was capable of producing a substantial excitation of motoneuronal activity (e.g., refs. 5, 6, 19, 24), whereas in anesthetized animals 5-HT only facilitated neuronal firing produced by other excitatory inputs (e.g., refs. 10, 13, 15, 16). This difference suggests that the importance of the excitatory serotonergic input to motoneurons may be easily underestimated in experiments utilizing anesthetics. The excitatory effect of 5-HT on XII motoneurons may be particularly significant because of their function as upper airway motoneurons [4]. In view of the known decrease in brainstem 5-HT neuron activity during REM sleep [17, 27], it is likely that the withdrawal of the endogenous excitatory effects mediated by 5-HT receptors revealed by this study may be relevant to the known decrease in upper airway patency during sleep [4].

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NK-1, but not NK-2, tachykinin receptors mediate plasma extravasation induced by antidromic C-fiber stimulation in rat hindpaw: demonstrated with the NK-1 antagonist CP-96,345 and the NK-2 antagonist Men 10207

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The effects of intradermal injection of CP-96,345 and Men 10207, selective antagonists for NK-1 and NK-2 tachykinin receptors, respectively, on the extravasation of plasma protein induced by antidromic stimulation of unmyelinated sensory fibers in the sciatic nerve was studied in rat hindpaw. Activation of unmyelinated fibers by antidromic sciatic nerve stimulation (1 Hz, 5 min) consistently evoked a localized plasma extravasation of Evans blue on the skin area of the hindpaw innervated by the sciatic nerve, which was not inhibited by intradermal injection of saline or Men 10207 (9 and 35 nmol). In contrast, CP-96,345 (3 and 9 nmol, but not 1 nmol), injected intradermally 15 min prior to nerve stimulation dose-dependently inhibited this response. Plasma extravasation induced by intravenously injected substance P was also inhibited by CP-96,345. Since CP-96,345 is a highly selective antagonist for NK-1 tachykinin receptors, it is suggested that the plasma extravasation induced by antidromic C-fiber stimulation and by systemically applied tachykinins is mediated by NK-1 tachykinin receptors.

Unmyelinated sensory fibers have a dual afferent/efferent function in response to noxious stimulation, the transmission of impulses into the CNS and participation in inflammatory reactions in the periphery through the release of chemical mediator(s). The extravasation of plasma protein through peri-capillary vessels into the extracellular space in peripheral tissues is a major component of neurogenic inflammation and there is compelling evidence that peptides of the tachykinin family may be involved in this response (see ref. 9 for review). The development of synthetic substance P (SP) analogs as specific antagonists for SP has provided the possibility to present conclusive evidence for the participation of tachykinins in neurogenic inflammation. However, interpretation of results obtained with previously developed tachykinin antagonists is hampered by the fact that they also possess pharmacological activities unrelated to the blockade of tachykinin receptors (see ref. 17). We have recently reported that intradermal injection of spantide II, a peptide tachykinin antagonist with negligible side effects [8], blocked plasma extravasation induced by antidromic C-fiber stimulation and intravenous (i.v.) SP, demonstrating a critical involvement of tachykinin receptor activation in this event [17]. These results, however, did not specifically identify the receptor subtypes involved in tachykinin-induced plasma extravasation, as spantide II is non-selective towards the NK-1, NK-2 and NK-3 receptors [8].

The present study was undertaken to examine the effect of intradermal injection of CP-96,345, a non-peptide NK-1 receptor antagonist [13, 15], on plasma extravasation induced by antidromic C-fiber stimulation in rat sciatic nerves and by i.v. SP. This compound labels NK-1 binding sites with high affinity in a number of species [13, 15]. Although its potency is lower in rodents [15], we and others have nevertheless found that CP-96,345 is an effective and selective antagonist of the NK-1 tachykinin receptor in rats [11, 14, 19], indicating the usefulness of this compound as a pharmacological tool in defining the role of the NK-1 receptor. For comparison, we have also studied the effect of Men 10207, a selective NK-2 receptor antagonist [12], on plasma extravasation induced by C-fiber stimulation and i.v. SP.

The experiments were carried out on female Sprague-Dawley rats weighing 200 g (Alab, Stockholm, Sweden). The rats were injected with 20 mg/kg guanethidine s.c.

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